# THE ECOLOGY OF BITING MIDGES (CULICOIDES SPP.) IN SCOTLAND.

by

Rachel Reuben.
B. Sc (Patna), M. Sc. (Delhi)



Thesis Presented for the Degree of Doctor of Philosophy
in the University of Edinburgh
from
Department of Zoology, The Ashworth Laboratory.

MAY, 1959.

[Degree conferred (in absentia) 24 October 1959]

ACKNOWLEDGEMENTS

I should like to thank Dr. D.S.Kettle for his help and encouragement at all stages of the work, and Dr. J.A.Campbell and Mr. E.C.Pelham-Clinton for the many helpful discussions I have had with them, and for lending me apparatus. I am also grateful to Dr. B.Woolf and Dr. L.J.Hale for invaluable help with the statistics. Mr. D.F.Cremer prepared all the photographic prints.

Most of the equipment used was obtained with a grant from the Earl of Moray Endowment, and Mr. A.Gall made some of it for me.

Finally I should like to thank the Staff at the H.F.R.O. farm at Lephinmore for their co-operation while the field work was being carried out.

-o-o-

CONTENTS

-o-o-

# INTRODUCTION

The genus Culicoides is widely distributed, being recorded from Europe, Asia, Africa, America and Australia. The importance of the genus is twofold. In several instances species of Culicoides have been incriminated as vectors of parasites of man and his domestic livestock. Sharp (1928) showed that Acanthocheilonema perstans, a human parasite in West Africa, develops in C. austeni. Sharp thought that C. grahamii was also concerned in transmission, but Henrard and Peel (1949) claimed that this species was more important as a vector for Dipetalonema (Acanthocheilonema) streptocerca. This has been confirmed by Duke (1954). Buckley (1934) observed the completed development of Mansonella ozzardi in C. furens. In Australia allergic dermatitis of horses is attributed to microfilariae of Onchocerca reticulata, carried by C. robertsi (Riek, 1954). In Britain Steward (1933) found that C. nubeculosis is a vector of Onchocerca cervicalis, associated with fistulous withers in the horse. Pathogenic virus diseases are also carried. Du Toit (1946) in South Africa recorded three infections of blue-tongue and one of horse sickness after intravenous injections of emulsions of Culicoides caught in the field.

The second and more obvious aspect of the genus is its nuisance value. Midges are painful biters. Jobling (1928) described the effect of a bite. It causes intense irritation and burning, which continue for a few minutes, but with more susceptible victims the pain lasts longer and the affected parts swell up to form red weals. Midges are so numerous as to constitute a major pest in some parts of the world. Biting rates of over 2,000 per hour have been recorded by Hopkins (1952) for C. grahamii in West Africa, and these are often exceeded in the West Highlands of Scotland by C. impunctatus.

C. impunctatus is the most numerous and troublesome species in Scotland, and is the main subject of the present study. It was first described from Belgium by Goetghebuer (1920), and is widely distributed in Europe. Cameron et al. (1946) have collected specimens from Sutherland, Ross and Cromarty, Inverness, Argyllshire, Perth, Stirling, Dumbarton, Renfrew, Lanark, Dumfries, Peebles, Midlothian, Angus, Kincardine, and Aberdeen.

In Scotland interest was focussed on the midge problem from 1946 when Cameron et al. produced a paper entitled Control of Midges, an Interim Report of the Sub-Committee of the Scientific Advisory Committee, in which they recommended the use of Dimethyl Phthalate as a midge repellent. This was followed in 1948 by A Second Report on the Control of

Midges, which was concerned with surveying the Scottish species and summarising the existing state of knowledge concerning their distribution, seasonal cycle and biting habits.

From this time onwards the study of bionomics proceeded simultaneously with that of taxonomy and control, since it was evident that an adequate knowledge of the habits of the midge was essential in conducting a programme of control. Hill in 1947 described the life-cycle of C. impunctatus, which she was able to rear through to the adult stage from eggs in the laboratory. In 1950 Parker published a detailed study of the effect of temperature on the hatching of eggs in the laboratory. He found that development was most rapid at 21°C., when the larvae hatched after 7 days, and also observed that hatching was delayed by dessication. The resistance of the egg to dessication increased with age. Parker did not rear C. impunctatus through a whole generation in the laboratory, and subsequent workers have not been able to do this. In fact it has only been possible to maintain a laboratory culture of two species of Culicoides. A culture of C. nubeculosus was established in Glasgow by Downes in 1947 and was successfully maintained till about 1955 (Megahed, 1956), and American workers have maintained a colony of C. variipennis (Jones, 1957). The fact that C. impunctatus could not be cultured in the laboratory has meant that physiological

studies on larvae and adults could not be made, and the emphasis has therefore been on field work. It has not been possible to confirm the results of field studies by laboratory experiments.

Although isolated observations on sites from which specimens had been obtained were available, for example those of Edwards (1939), and Hill (1947), the species were not grouped on the basis of larval habitat until 1952, when Kettle and Lawson classified breeding sites under six headings. These were: (i) Bogland; (ii) Fresh water marsh; (iii) Swamp; (iv) Mud; (v) Salt marsh and (vi) Dung. C. impunctatus, in association with C. truncorum, C. heliophilus, C. albicans, and C. obsoletus, occurred in bogland breeding sites. These were characterised by the growth of Sphagnum spp., Polytrichum spp. and Juncus articulatus. These plants served as good indicators of potential breeding sites for C. impunctatus, which must be taken into account in work on adult bionomics.

Hill (1947) showed that C. impunctatus passed the winter as a fourth instar larva. Pupation started in April and the first adults appeared at the end of the same month. There was a steady increase in numbers throughout May, and after a peak in June there was a decline. No adults were caught after August. These results were obtained in

Lancashire. Parker (1949) working at a site by Loch Lomond, found that the season extended from the beginning of June to the end of August, with a maximum in early July. He pointed out that the difference between his results and those of Hill were probably due to the fact that emergence tended to take place later in the north than in the south. Hill's method of estimating numbers was to capture all adults alighting on a black cloth within a period of an hour at a time related to the time of sunset. Parker used a sweepnet in his studies. Kettle introduced the use of the sticky trap to the problem of estimating midge population. A grease-treated celluloid sheet is fastened round a metal cylinder, which is fixed to a post at a standard height above the ground. The celluloids are changed at weekly intervals, and the number of midges trapped in the grease over that period are an estimate of their abundance during that week. The use of this trap makes it possible to compare numbers at several different sites for the same week, and in addition eliminates the human factor and standardises trapping conditions. Working at a site near Loch Lomond (not the same as that where Parker worked), and using sticky traps, Kettle found that in the years 1948 and 1949, C. impunctatus showed a bimodal seasonal distribution with peaks in June and July. A bimodal distribution had previously been noticed in 1947 by Currie (unpublished), who was using a sweepnetting technique. Currie was working

at the same site as Parker, and in the same year. The difference between the time-frequency distributions obtained by the two workers is explained by the fact that Parker was away on holiday at the time when Currie found a second peak Weather factors did not seem to be sufficient to account for the fall-off in numbers in the middle of the season, and Kettle put forward the suggestion that C. impunctatus might be composed of two biological races with different times of emergence. However, later work by Parish (unpublished), at Loch Maree and elsewhere in Scotland, showed that the two peaks did not invariably appear in the seasonal cycle, single peaks being obtained in some years for some sites.

In his paper of 1949 Parker also described the daily cycle of C. impunctatus. Maximum numbers were captured between three hours before to one hour after sunset, and there was a second smaller increase in activity at sunrise, and they were virtually absent at other times of day. A similar pattern of activity was described for C. nubeculosus by Downes in 1950 and for C. grahamii in West Africa by Nicholas in 1953. Nicholas also found that C. austeni in the same area showed a different type of cycle, being active all night after the light intensity had fallen below a certain threshold. Nothing is known about the mating habits of C. impunctatus apart from Downes' statement in

1955 that he observed four or five pairs falling through the air on one occasion. From this he concluded that mating took place during swarming flight. He was however, able to study mating behaviour in greater detail on C. riethi and C. nubeculosus and observations on some other species seemed to show that they were similar. Male swarms formed in relation to definite swarm-markers, cowpats, bracken fronds, etc., depending on the species. Females flew into the swarms attracted by the same markers as the males, and were recognised by the males, which swarm with antennal setae erected. Mating pairs fell out of the swarm. Subsequently the male rejoined the swarm but the female did not. In addition C. riethi and C. nubeculosus were able to mate in confined spaces. Another aspect on which very little work has been done is the feeding behaviour of Culicoides.

Cameron et al. stated in 1946 that in their survey of midges in the West of Scotland C. impunctatus made up 76 % of the total population, but gave 90 % of all biting records. In the Second Report in 1948 they included this species among those found feeding on cattle. Hill (1948, unpublished) performed precipitin tests on the stomach contents of several species of Culicoides. She concluded from these that C. impunctatus was the most important species attacking man, which confirms the findings of the other workers. Hill's

findings must however be regarded as preliminary since collections were made in buildings, where there could be expected to be a bias. No studies on the gonotrophic cycle and the development of eggs or on fertilisation have been made on British species. Nicholas (1953) described these for C. austeni and C. grahamii using methods which were standard in mosquito work.

In 1951 Kettle published a paper on the spatial distribution of C. impunctatus. He found that there was a movement away from the breeding site in the direction of the prevailing wind, and that a linear relationship could be found between the logarithm of the catch and the distance from the centre of breeding. Having obtained the formula for the regression the average distances flown by males and females were calculated. These were 79 yards for males 81.4 yds for females. The vertical distribution of the catch varied according to the location of the traps. In woodland where the canopy was about 20 feet C. impunctatus did not fly higher than 10 ft. On open moorland the catch was evenly distributed between the three heights at which trapping was conducted. Where there was a herb layer however, the 2 ft. trap caught more than the 6 ft. Bracken did not provide suitable shelter, since of those traps situated among bracken the 2 ft. traps caught less than the 6 ft. ones.

The distribution of males and females was also significantly different, and this appeared to be the result of a difference in behaviour between the sexes. Differential behaviour is also thought to account for the peculiar sex ratios obtained for many species of Culicoides, including C. impunctatus, by various trapping methods. Kettle reviewed these findings, which are scattered through the literature, in 1955, and pointed out that for 26 British species the sex ratios obtained by rearing adults from larvae are normal.

This brief survey shows that a considerable background of knowledge already exists concerning the habits of C. impunctatus. The first part of the present thesis represents an attempt to fill in a few of the gaps in this background, particularly with regard to egg development. The second part concerns the effect of climatic factors on the activity of the midge.

It was soon realised that in order to relate activity as measured by trapping to population density, it was necessary to know the effect of climatic conditions on activity. Kettle (1957) made a 24-hour study in which collections were made simultaneously with measurements of light intensity, temperature, humidity, and wind-speed. From this it emerged that C. impunctatus was most active under conditions of low light intensity, fairly high humidity and calm.

The difficulty of maintaining a culture of Culicoides in the laboratory has probably been responsible for the fact that no experimental studies have been made on the responses of the genus to physical factors. On the other hand much work has been done on the responses of mosquito species. For example, Martini and Teubner (1933, quoted by Bates, 1949) worked on the responses of Aedes aegypti and Anopheles maculipennis, and Thomson (1938) on those of Culex fatigans, to gradients of temperature and humidity. Platt et al. (1957) described the effects of temperature, humidity and light on Anopheles quadrimaculatus. The interpretation of ecological findings in the light of such studies is not easy. The early work on the subject has been reviewed by Bates in 1949 and by Thomson in 1951. Daily activity cycles have been described for many species, and attempts have been made in some cases to explain these in terms of the microclimate. Important among these is the series of researches made on the biting cycles of the West African species by Haddow and others. In 1945 Haddow put forward the view that the biting cycle of Anopheles gambiae which is nocturnal and Eretmapodites chrysogaster which is diurnal, were simply related to the daily temperature and humidity cycle. The former became increasingly active as the temperature dropped and humidity rose, while the latter

showed the opposite reaction. It was thought that this hypothesis could be extended to explain the biting cycles of other species of mosquitoes. Later studies showed that this was not so. By 1956 Haddow had abandoned this hypothesis in favour of the view that unfavourable microclimatic conditions could inhibit biting, but that the basic pattern was related to the gonotrophic cycle, females at different stages biting at different times. This view had already been put forward by Lumsden in 1952, who suggested that the wave of activity following the release of inhibition represented older females ready to bite, whereas younger females might require a period for maturation before feeding.

Daily cycles are known for other groups of insects. Of these, that of the aphid has been extensively studied at Rothamsted Experimental Station over a number of years. Both laboratory and field studies have assisted a better understanding of the factors contributing to the daily pattern of activity than is at present available for any other insect. Early attempts to account for the changing numbers of aphids over crops were based on the hypothesis that activity was directly related to changing weather conditions. Davies (1939) for example, stated that the conditions for aphid migration were a temperature above 70°F., a low

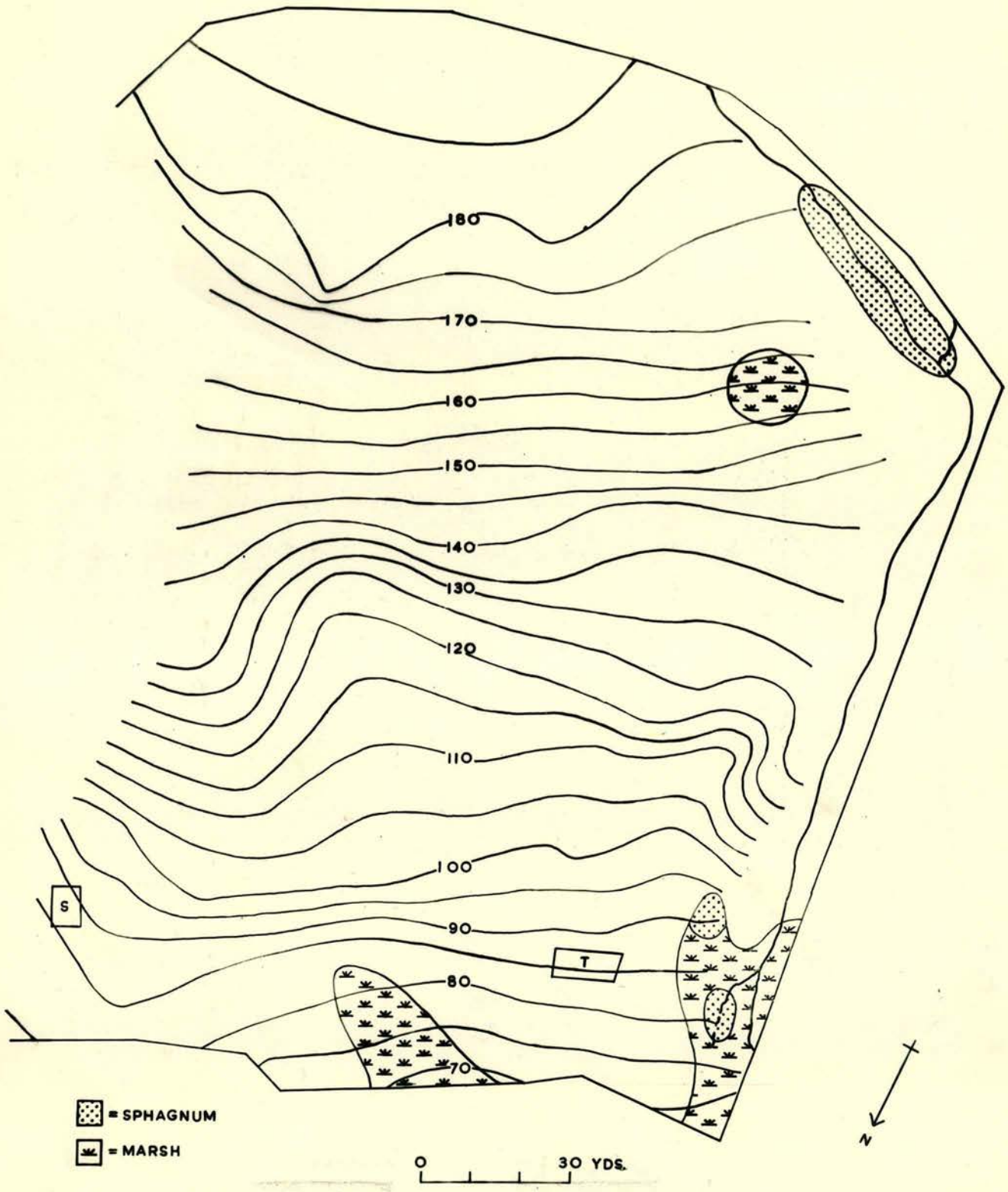
humidity, and a wind-speed of less than 5 m.p.h. Thomas and Vevai (1940) confirmed these findings. However Johnson (1952) showed that the relation between catch, wind-speed and temperature were nonsignificant when multiple regression methods were used. He therefore put forward a new hypothesis to the effect that flight activity was a reflection of the rhythm of moulting and maturation, and that this was only secondarily affected by climatic factors. Further investigations were carried out to confirm this. The moulting rates of nymphs of Aphis fabae in different parts of a bean field were estimated simultaneously with measurements of wind-velocity, temperature and humidity, while suction-traps sampled aerial density immediately above. It emerged that there was an obligatory resting period between moulting to the alate form and flight, and this was termed the teneral period. Aphids during the teneral period could not take flight even when conditions were suitable. Taylor (1957) showed both by field and laboratory studies that the length of the teneral period depended on temperature, and that there was a "thermal constant" which had to be completed before an aphid was ready to fly. The relation between developmental velocity and temperature was shown to be a semi-logistic curve. It follows that

it is possible to calculate the length of the teneral period under a particular set of temperature conditions. Johnson and Taylor (1957) showed that aphids maturing during the hours of darkness were inhibited from flight, and tended to accumulate. As soon as light intensity and temperature rose above their respective thresholds, these took flight, giving rise to the early morning peak of activity. The numbers of aphids taking off thereafter showed an immediate drop, but there was a steady rise afterwards as higher day temperatures caused teneral periods to become shorter. Failing light and dropping temperature brought activity to an end by nightfall. Thus the bimodal activity well known for aphids was explained. Further, taking a case where the moulting rates and the temperature fluctuations for the relevant period were known, they reconstructed theoretically the pattern of activity which was to be expected. This showed a very good agreement with the activity which was actually observed. It would seem therefore that with aphids there is a fairly satisfactory understanding of the factors contributing to the hourly fluctuations in aerial numbers.

In the case of aphids, as well as in the mosquito examples discussed earlier, the recent trend has therefore

been to regard the daily cycle of activity as basically determined by developmental phenomena and modified by flight behaviour. The work described in the second part of this thesis was designed as a preliminary attempt to explain the changing numbers of Culicoides impunctatus in these terms.

-o-o-


180
170
160
150
140
130
120
110
100
90
80
70
S
T
= SPHAGNUM
= MARSH
0
30 YDS.
N

## DESCRIPTION OF THE TRAPPING SITE.

The experimental work described in this thesis was carried out at a field-station at Lephinmore, Argyllshire. This is a research farm on Loch Fyne side, belonging to the Hill Farming Organisation. It was by kind permission of the Director, Mr. A.R.Wannop, O.B.E., that facilities were made available for carrying out the work. The West coast of Scotland was chosen because C. impunctatus was known to be abundant there, accounting for 76 % of the total Culicoides population (Cameron et al., 1946), and Lephinmore was selected in particular because mains electricity was available at the site. The Moredun Institute of Animal Diseases had been trapping Culicoides there over a number of years, using suction traps of a type developed by Johnson (1950) and improved by Taylor (1951). The actual trapping site was an enclosure 17 ft. by 34 ft., in a large field south of the steading, and was fenced off with wire-netting and barbed wire to exclude animals. A cable had been taken from one of the mains electricity poles by the road to the site, a distance of about 50 yds. The site had trees on two sides of it, north and west, 15 and 25 yds. away respectively, but was open on the east and south.

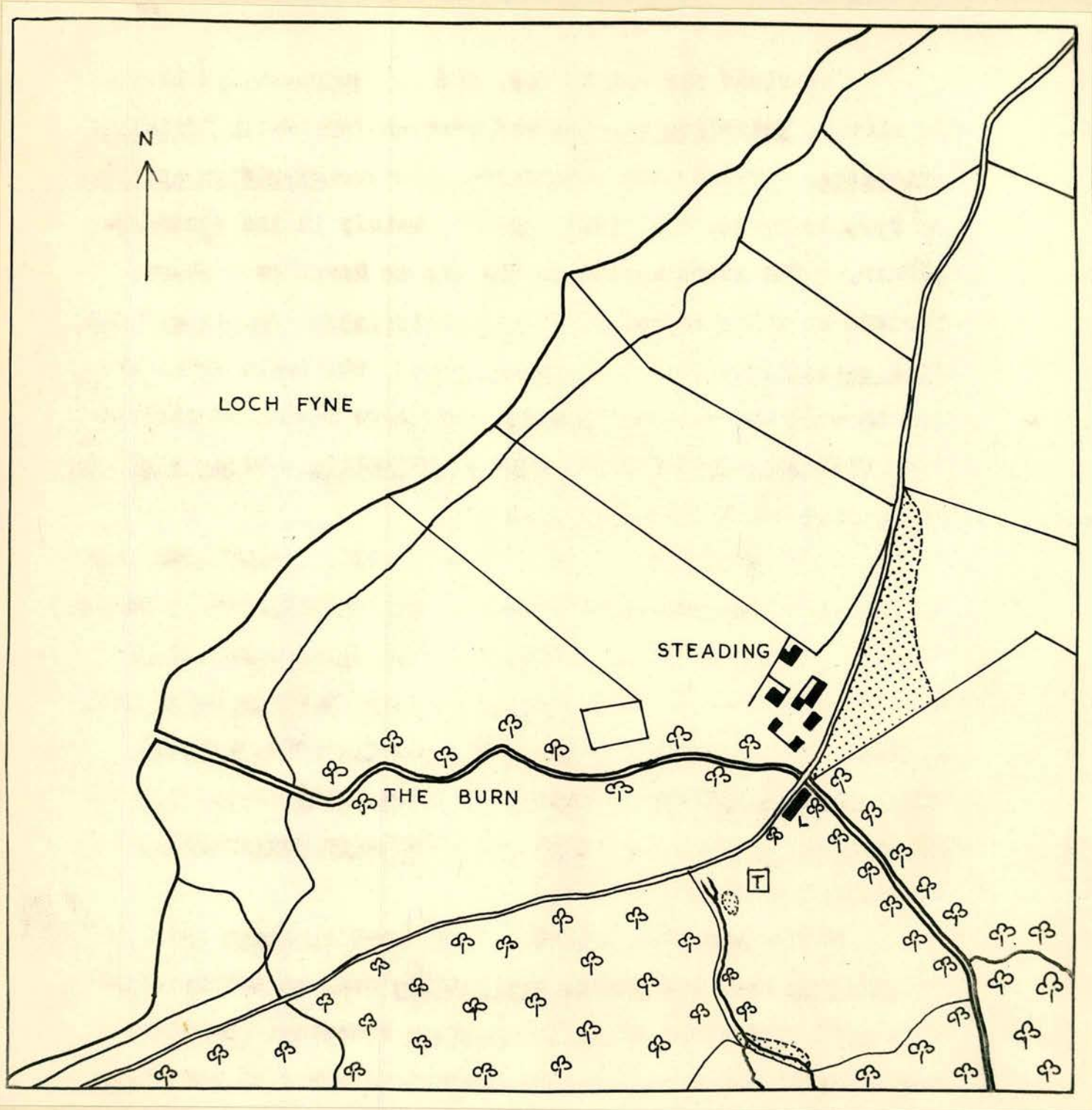



Figure 2. Map showing position of trapping site (T) in relation to steading. Breeding site indicated by dotted areas. L = Laboratory.

The field was mainly dry, and its vegetation consisted of clover, Trifolium repens, and grasses, typically Cynosurus cristatus. There were patches of bracken, Pteridium aquilinum further up the hill (see fig. 1 ) mainly in the south-east corner. The areas marked on the map as Marsh were characterised by the presence of Juncus articulatus, Juncus effusus, Iris pseudacorus, and Ranunculus repens, while the areas dotted in were those where Sphagnum spp. were found, in association with Polytrichum spp., Juncus articulatus, Carex echinata, Iris pseudacorus and other species.

A stream ran down the western border of the field, and to the east the ground fell away sharply to the burn. Beyond the burn to the east was a strip of boggy ground (see fig. 2). Besides drier patches where Holcus mollis, Festuca ovina tenuifolia, Erica tetralix and Calluna vulgaris grew, Sphagnum spp., Juncus articulatus, Juncus effusus, Carex spp., Orchis ericetorum, Orchis purpurella and Eriophorum angustifolium were found in this strip.

Kettle and Lawson (1952) found that Sphagnum spp., Polytrichum spp. and Juncus articulatus when in association, were good indicators of C. impunctatus breeding. On this basis there were three potential breeding sites on the farm (see fig. 2 ); one on top of the slope and in the south-west corner of the field, about 160 yds. away from the trap-

ping site, the second about 20 yds. to the west, and the third beyond the burn to the east, about 250 yds. away. The relative extents of these three breeding sites is evident from figure 2. It was thought earlier (Kettle, 1951) that the average flight-range of C. impunctatus was under 100 yds. It has now been established that the species can fly at least half a mile (Kettle, in press). This means that all three sites could contribute to the suction trap catch, and it seems likely that the majority of the specimens came from the bogland strip beyond the burn, since the other sites are very restricted.

-o-o-

# PART I

## MATERIALS AND METHODS.

The most abundant species at Lephinmore from May to September is C. impunctatus, so this species was used in all the investigations. In addition fertilization studies were made on C. heliophilus and observations on obsoletus, circumscriptus and riethi have been recorded. The two last named species were obtained as larvae from a site at Airth, at the south end of Kincardine Bridge, described by Downes in his paper in 1955. They were reared to maturity in the laboratory.

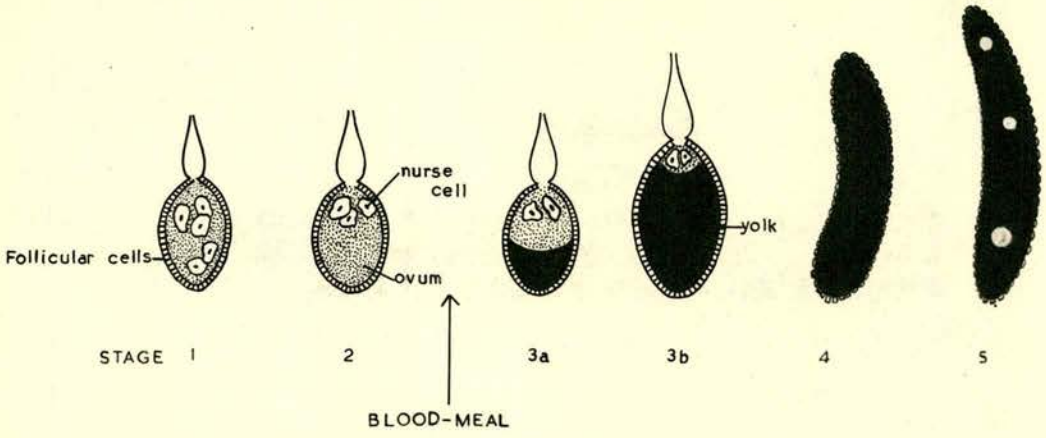
Dissections were made on wild-caught female impunctatus, heliophilus and obsoletus, to elucidate the stages of development of the ova. The specimens were taken from suction-trap collections (described in greater detail in Part II). The later stages of ovary development, which were not common in suction-trap collections, were obtained by keeping wild-caught, blood-fed specimens in tubes in the laboratory at room temperature and feeding them on sucrose solution on alternate days. They were dissected at appropriate intervals of days. The abdomen of each specimen was detached and placed in a drop of physiological (0.9 % ) saline on a slide while the thorax was dry-mounted and stored for further reference.

The dissection of the abdomen was carried out under a low power binocular. The anterior segments were held down with one needle and with another the terminal ones were loosened from their connections and gently drawn away. The reproductive system usually came away with the terminalia without distortion and the alimentary canal could then be removed.

A similar dissecting technique was used when measuring the ampullae and examining spermathecae, but for these studies live specimens were required and sweepnet collections were made. The living midge was transferred with a damp camel-hair brush to a slide and decapitated in saline immediately before dissection. Measurement of the ampullae was carried out using an eyepiece micrometer with a monocular microscope, oc. 6 obj. X40. At this magnification spermatozoa, when present, could be seen swimming actively round inside the spermatheca. As a confirmatory measure, however, the spermatheca was generally mounted under a coverslip and crushed. This released the sperm into the surrounding liquid where they could be seen more clearly.

In an attempt to find the resting places of adult midges a second type of suction trap devised by Johnson et al. (1955) for extracting arthropods from herbage, was used. The apparatus is described in more detail in the relevant section of the text.

-o-o-


Follicular cells
nurse cell
ovum
yolk
STAGE 1
2
3a
3b
4
5
BLOOD-MEAL

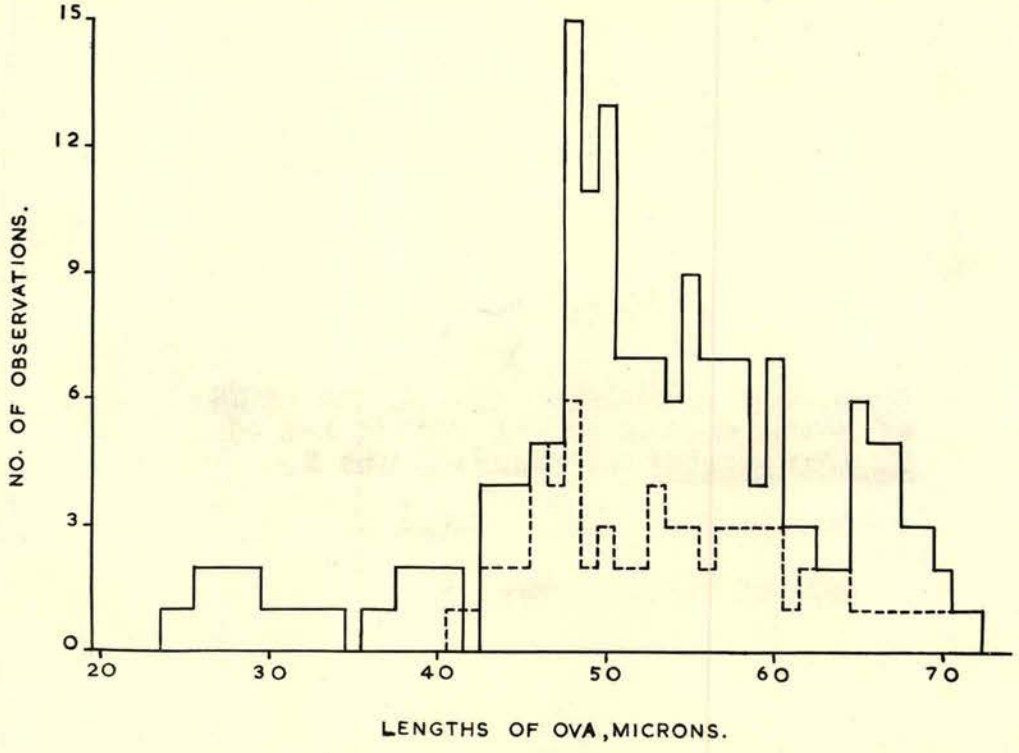

NO. OF OBSERVATIONS.
0
3
6
9
12
15
20
30
40
50
60
70
LENGTHS OF OVA, MICRONS.

Figure 3

The stages of development of the ova.
Stages 1, 2 and 3 approximately X 30.
Stages 4 and 5 approximately X 15.

Figure 4

Frequency histogram showing the range of measurements of individual ova of *C. impunctatus* at Stages 1 and 2.

Continuous line: Stage 1.

Broken line: Stage 2.

# RESULTS

## Egg Development

The development of the egg-follicle in anopheline mosquitoes was described by Christophers (1911). For convenience he divided it into five arbitrary stages, and this classification has been followed by subsequent workers. Ovulation in Culicoides was studied in two species, impunctatus and heliophilus, specimens from suction traps and sweepnet collections being used. Development of the ova of C. austeni has been described by Nicholas (1953), but he did not relate the various stages with comparable stages in mosquito development.

The course of development of the ovum was found in the present investigation to be very similar to that of Anopheles. The follicle began as a group of undifferentiated cells, the outermost of which soon arranged themselves to form a distinct layer of Follicular cells. One of the cells in the central mass became distinct from the rest and took up a position at the lower end of the follicle to become the Ovum. The remainder were the Nurse cells, which, as the ovum enlarged, were pushed further and further towards the upper end of the follicle. They could still be seen at the upper end of the egg when the latter was almost mature (fig. 3 ). With the

increase in size of the egg, yolk granules were deposited around the nucleus and gradually obscured it. The egg finally lost its oval shape and became elongated. Just before laying certain clear spaces appeared in the yolk which were taken to consist of reserve food material of a different refractive index to the rest of the yolk.

Not all the changes described by Christophers for Anopheles were obvious, however. For example, the distinction between the stage of fine yolk deposition and the appearance of coarse yolk granules in the cytoplasm of the egg was not clear. This was possibly due to the much smaller size of the ovum of Culicoides. The appearance of fine yolk granules is the criterion of the onset of Stage 2 in Anopheles, but since this was not clear in Culicoides it was decided not to follow Christophers but to redivide the developmental cycle into stages on the basis of changes which were more striking.

The sequence of changes observed was as follows:

Stage 1. Follicle consisted of undifferentiated cells. Follicle and central cells became distinguishable.

Stage 2. Nucleus of ovum became differentiated from nurse cells.

Stage 3. (a) Yolk granules appeared in the cytoplasm of the ovum. Yolk obscured the nucleus of the egg.

(b) Ovum grew in length.

Stage 4. Ovum became elongate instead of oval.

Stage 5. Clear spaces appeared in yolk.
Egg was laid.

These stages were comparable to the following stages in Anopheles.

| | Culicoides. | Anopheles. |
|---|---|---|
| Stage: | 1<br>2 | 1 |
| | 3 | 2<br>3 |
| | 4 | 4 |
| | 5 | 5 |

Within the ovary of a single specimen the ova, though at the same stage of development, were variable in size. The lengths of five ova were taken for each individual and the largest and the smallest of the five measurements have been recorded in Tables 1 and 2. The five measurements were not randomly made, selection having been exercised to include the largest and smallest ova in the ovary. Where all the ova measured were the same length only one figure is recorded.

The range in length of ova in specimens at the same stage of ovary development was as follows:

C. impunctatus

| Stage | 1 | 2 | 3a | 3b | 4 | 5 |
|---|---|---|---|---|---|---|
| Range (μ) | 24.0-72.0 | 40.8-72.0 | 76.8-81.6 | - | 120.0-220.8 | 374.4-460.8 |
| Number of specimens | 33 | 13 | 1 | 0 | 5 | 2 |

C. heliophilus

| Stage | 1 | 2 | 3a | 3b | 4 | 5 |
|---|---|---|---|---|---|---|
| Range (μ) | 33.6-74.4 | 38.4-81.6 | 48.0-79.2 | 76.8-103.2 | 84.0-172.8 | - |
| Number of specimens | 15 | 7 | 4 | 3 | 6 | 0 |

It will be seen that there is considerable variation in the sizes of ova at any one stage, and a certain amount of overlap between the stages. There does not seem to be any significant difference in the sizes of ova at Stages 1 and 2. This emerges from figure 4 which is a frequency histogram of lengths of ova at Stages 1 and 2. Where there was a range of size within a single ovary, e.g. 38.4 μ to 43.2 μ the individual was entered in the columns corresponding to 38, 39, 40, 41, 42 and 43. From Stage 3 onwards however, there is a steady increase in length.

The Rôle of the Blood-meal.

C. impunctatus can occur in astronomical numbers on

bog and moorland far from human habitation. The fauna of such situations consists of small mammals, birds and the occasional sheep, and it is difficult to see how these could provide a blood-meal for a large enough section of the population to maintain numbers more or less constant from year to year. It was therefore thought that a blood-meal might not be obligatory in the gonotrophic cycle and that it would be possible to demonstrate autogeny in this species.

Blood-fed midges were caught with a sucking tube on a north-facing window in a henhouse, where they had presumably been feeding on the hens at night. They were kept in tubes at room temperature, and dissected after an interval of days. Unfed specimens in tubes were also kept, under identical conditions. Both series were fed on alternate days on sucrose solution soaked on filterpaper.

The results of the dissections were as tabulated on page 25.

. . .

It appeared therefore, that a bloodmeal was necessary for the completion of the gonotrophic cycle, and that development would not proceed beyond Stage 2 in its absence, i.e. that yolk deposition would not take place. This agrees with the findings of Pfeiffer (1945) who showed that yolk formation

Results of Dissections.

| | Interval between capture and dissection (days). | Ovary Stage 1 | 2 | 3a | 3b |
|---|---|---|---|---|---|
| Unfed. | 1 | 2 | - | - | - |
| | 5 | 2 | 4 | - | - |
| | 7 | 4 | 3 | - | - |
| | Total: 15 specimens. | | | | |
| | % showing yolk deposition: 0 | | | | |
| Bloodfed. | 1 | 1 | 1 | - | - |
| | 5 | - | 5 | 2 | - |
| | 7 | - | - | - | 4 |
| | Total: 13 specimens. | | | | |
| | % showing yolk deposition: 46.2 % | | | | |

in Melanoplus differentialis was dependent on the activity of the corpora allata, and Harlow (1956) who found a similar dependence in Protophormia terrae-novae. Wigglesworth (1936) demonstrated that the activity of the corpora allata depends on its nutritive state, and that egg development halts until an adequate meal is taken, when yolk deposition can occur. In Aëdes aegypti however, the resting stage is Christophers' Stage 2 (Gillet, 1956), when yolk deposition has begun. The fact that the development of oocytes beyond the resting stage was hormone controlled was demonstrated for Anopheles maculipennis by Detinova in 1945, by ligaturing specimens behind the corpora allata at different time intervals after the blood-meal. Similar experiments by Clements (1956) on

Culex pipiens and Gillet (1956) on Aëdes aegypti showed that the same was true for these species also.

The results obtained for C. impunctatus do not necessarily mean that this species is anautogenous. Autogeny is known in the Culex pipiens complex where it has been extensively worked on. The ability to produce eggs without a blood-meal is influenced by factors other than genetical. Tate and Vincent (1936) found that in C. molestus which is autogenous, on an average 60 % of the females laid, but fewer when larval feeding was inadequate. Crosses between molestus and pipiens showed that autogeny always appeared in the F2 generation and sometimes in F1 (Tate and Vincent, 1936) and Laven (in Mattingly et al., 1951) considers the character to be unifactorial and recessive to anautogeny. This means that autogeny may appear spontaneously in an autogenous population which is nevertheless heterozygous for this gene. This has been known to occur (Vincent, 1933, quoted in Mattingly et al., 1951). Thus to determine whether a species is autogenous or not several generations of inbreeding under known rearing conditions would be required.

Specimens of C. circumscriptus reared from pupae obtained from Kincardine Bridge could not be induced to develop eggs. This confirms the findings of Becker (1956).

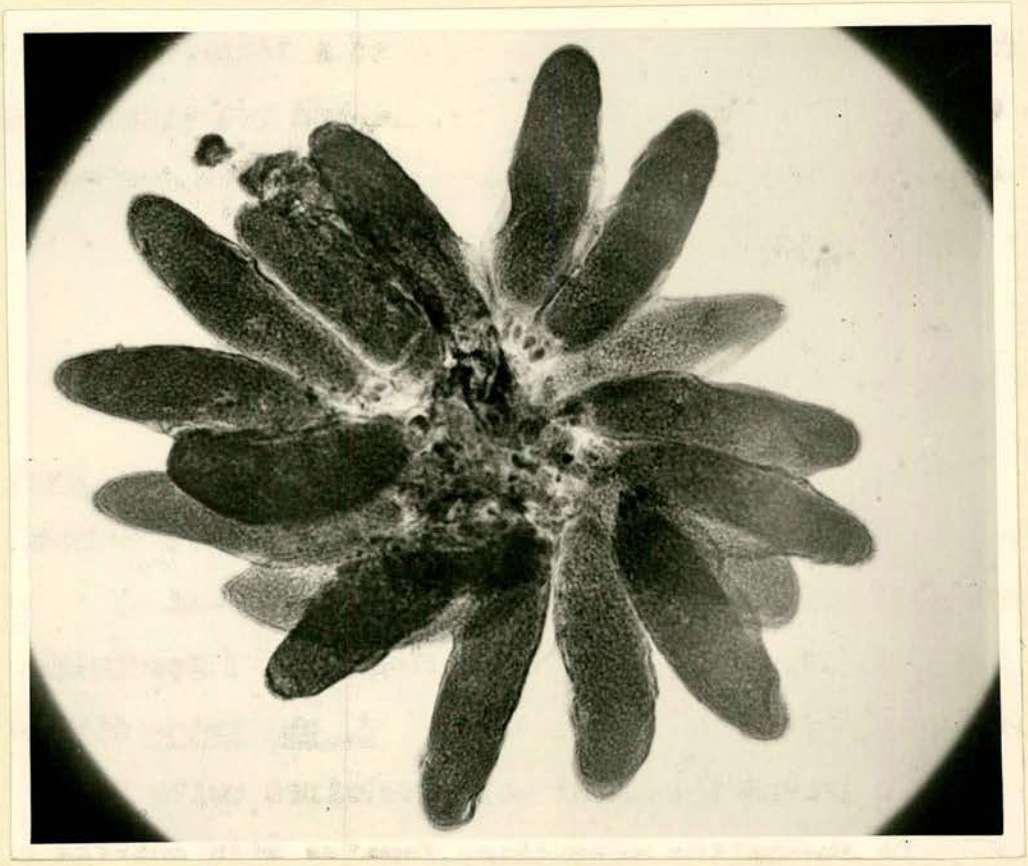


Figure 5. Mature ova of C. riethi: X 20.

Becker failed to induce this species to feed on mammalian or avian blood, or on cockroach nymphs.

C. riethi reared from pupae from the same source was found to be autogenous. Four specimens, which had not fed, were found on dissection to be gravid. They emerged between 25/12, and 28/12/1956 and were obviously gravid on 4/1/1957. One was dissected immediately while the others were fed on sucrose solution and kept at a temperature of 23° - 25° C. until 13/1 when they still had not oviposited. They were then dissected. Figure 5 shows ripe ova from one of the specimens.

Sampling Herbage by Suction.

The aerial population of C. impunctatus and C. heliophilus, as sampled by the suction-trap, was almost entirely composed of unfed females with ova at Stages 1 and 2. Very early Stage 1, Stages 3, 4 and 5, and blood-fed specimens, appeared very rarely in the catches. C. obsoletus differed, blood-fed and gravid specimens being obtained quite frequently. In anopheline mosquitoes females with ovaries at early Stage 1 are those which are less than 24 hours old (Christophers, 1911). The infrequency of this stage in catches of Culicoides would be explicable if it were

similarly short-lived, since the life-span of C. impunctatus at least, as will be shown later on, is fairly long in comparison with the suggested duration of early Stage 1.

The absence of blood-fed midges at the later stages of ovary development must be due to the fact that they are not active fliers. Very little is known about the resting places of adult Culicoides in nature. Several species, pulicaris, punctatus, obsoletus, scoticus, stigma, and occasionally impunctatus, were found at the base of whitebeam trees outside the Zoology Department at Glasgow (Kettle, personal communication). Nicholas (1953) took resting engorged C. austeni in buildings where they had been feeding, and on dead banana and plaintain leaves hanging round the stems of the plants. Sweepnet and suction tube collections in buildings and on vegetation failed to reveal the resting places of C. impunctatus. Jobling (1953) found adults of C. vexans resting at grass-roots, and in the soil to a depth of 5 inches on dry days. It is impossible to sample habitats like these with a sweepnet and collection of resting midges therefore presented a problem.

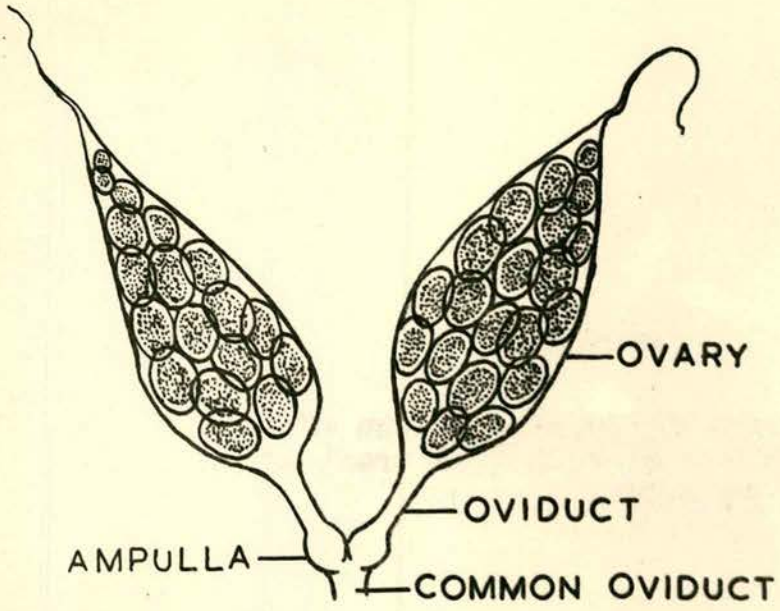
Johnson et al. (1955) described a method of extracting small arthropods out of vegetation which seemed to be the solution. The apparatus was essentially a suction-pump.

A rubber nozzle at the end of a flexible hose led to the collecting-bag, which was housed in a metal tube attached to the intake of an electric blower. The prototype of this apparatus was borrowed from Rothamsted for part of the summer of 1956. It was run off the mains and by the use of a 200 yard cable a very large area was sampled.

The trap was used on various types of habitat - open grassland, bog, bracken, holes in walls, banks, etc. - but with very disappointing results. When used on dry situations, although other arthropods were picked up satisfactorily, no midges were found. This could not be taken as proof that they are not resting on open dry grassland because there was evidence that Chironomids and other small Diptera were being damaged and in some cases becoming unrecognisable during their passage through the hose. Extractions were also made with the hose removed and the nozzle attached directly to the mouth of the tube, but this made the apparatus rather unmanoeuvrable.

When used on the bracken and rushes at the edge of a breeding-site on the opposite bank of the burn to the laboratory (see fig. 2 ), the trap found midges on two occasions only. On these occasions the hose had been removed.

5/6/1956, 2 p.m. 1 gravid ♀ C. heliophilus. Juncus, Ranunculus.


OVARY
OVIDUCT
AMPULLA
COMMON OVIDUCT

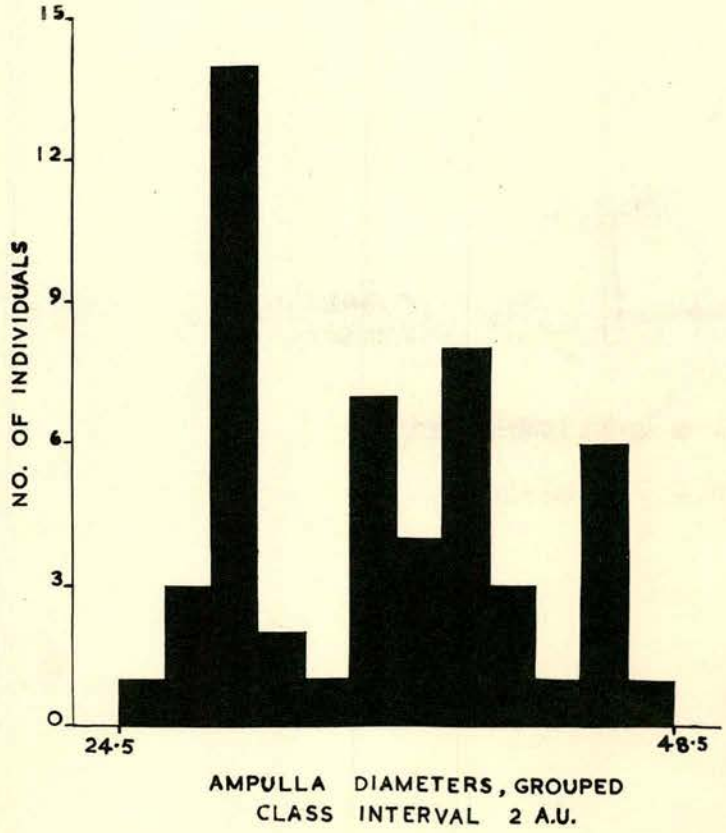

15
12
9
6
3
0
NO. OF INDIVIDUALS
24·5
48·5
AMPULLA DIAMETERS, GROUPED
CLASS INTERVAL 2 A.U.

Figure 6

Diagrammatic representation of the female reproductive tract of C. impunctatus.

Figure 7

Frequency histogram representing the range of ampulla diameters measured.

A. U. = arbitrary units.

1 a.u.= 2.4 microns.

| | | |
|---|---|---|
| 20/7/1956, 12.30 p.m. | 4 ♂ C. impunctatus. | Juncus, Ranunculus. |
| | 1 gravid ♀ C. pallidicornis. | Juncus, Ranunculus. |
| 4.30 p.m. | 4 unfed ♀ C. impunctatus. | Bracken and long grass. |

On damp ground the method failed entirely. Water was sucked up and any small insects that were taken were completely unrecognisable. This was unfortunate as it is highly probable that midges choose resting places on boggy ground.

Ampulla Measurements.

Mer (1932) described the changes which take place in the size of the common oviduct of Anopheles sacharovi which make it possible to distinguish between nulliparous and multiparous females. Since then many other workers have used this character as a criterion for aging wild-caught females of several species of Anopheles (see the review by Davidson, 1955). Measurements were made on the oviduct of C. impunctatus in the hope that similar changes in size would emerge. It was found that, as in Anopheles, the proximal part of the common oviduct showed two distinct dilations, the ampullae (fig. 6). Only the diameter at right angles to the main axis was measured, since the other was not very well-

defined. When the two ampullae were unequal in size, the larger of the two measurements was used. Davidson (1955) emphasizes the fact that the increase in ampulla size being a continuous process, only females at the same stage of the gonotrophic cycle should be compared if clearcut differences in size between nulliparous and multiparous specimens are to emerge. All the specimens of C. impunctatus used in this study were caught flying and had ovaries at Stages 1 or 2.

It was found convenient to work directly in ampulla diameters rather than in ampulla areas as Mer did. Diameters were expressed in arbitrary units equivalent to 2.4 $\mu$ and these have been used in drawing up the frequency histogram in figure 7. For actual measurements see Table 3. The ampulla diameters measured ranged from 60.0 $\mu$ to 112.8 $\mu$. This may be compared with Anopheles gambiae, where diameters ranged from 109.0 $\mu$ to 340.0 $\mu$ (Davidson, 1955). Davidson's dissections were made in distilled water, which caused the ampullae to swell a little. It is possible that in distilled water a wider range of diameters would have been obtained for Culicoides, but the degree of distortion seemed to be excessive, so saline was used.

On comparing this frequency distribution with a normal distribution a chi-square ($X^2$) of 22.01 is obtained. For

7 degrees of freedom (n) this corresponds to a probability (P) of 0.01. A normal distribution cannot, therefore, be fitted to all the data. If both nulliparous and parous females, having different ampulla parameters, are included in the populations sampled, one would expect to find at least two groups contained in the data. The first five classes appeared to form a separate entity, which, when tested for normality gave a chi-square of 5.66. For 2 degrees of freedom, a probability within the 0.1 - 0.08 range was obtained. The deviation from normality was therefore insignificant. A similar test for normality for classes 5 to 12, which were taken to constitute the second group, again showed no significant deviation ($X^2 = 4.07$, $n = 4$, $P = 0.5 - 0.3$); see note to Table 3. Class 5 has been included in both groups since it is not obvious to which it belongs. Group I, therefore, includes females having ampulla diameters between 25 and 34 units (60.0 $\mu$ and 81.6 $\mu$), and Group II those with diameters between 33 and 48 units (79.2 $\mu$ and 115.2 $\mu$). Individuals with ampulla diameters from 79.2 $\mu$ to 81.6 $\mu$ cannot be assigned with certainty to either group.

It seems more satisfactory therefore to explain the distribution of frequencies represented in figure 7 in

terms of two normal distributions rather than a single one. There is no biological evidence that the two populations correspond to two age-groups, and the choice of class 5 as a dividing point is thus somewhat arbitrary. It was unfortunately not possible with the inadequate facilities available at the fieldstation to rear adults from pupae; and all the specimens which were induced to oviposit in the laboratory died immediately afterwards. It was therefore not possible to measure the ampullae of females of a known age. Only one of the females dissected had two eggs from a previous egg-laying retained in the ovary of one side. This specimen had an ampulla diameter of 38 units (92.2 $\mu$), thus falling within Group II.

Though the evidence produced is in accordance with the hypothesis that there is, as in Anopheles, an increase in the size of the ampulla with age, dissections on laboratory reared specimens of known age are necessary to confirm this.

The specimens dissected in this study were randomly selected from sweepnet collections. It is interesting to note that Group II females are more numerous than Group I, there being 31 and 21 individuals respectively to each group. If the two groups do in fact correspond to the two kinds of females this means that in the latter half of

August when these dissections were made, only 40.4 % of the population had not oviposited before. The remainder had been through at least one gonotrophic cycle already. This could indicate that towards the end of the season the importance of the newly emerged section of the population was dwindling, and that most of the females flying had emerged some time previously and had returned for another blood-meal. To make this possible an appreciable section must survive the first egg-laying, and the average length of life must be several weeks.

Fertilisation Studies.

Wild-caught females of two species, impunctatus and heliophilus, were dissected in saline and their spermathecae examined for the presence of sperm. Both these species have two functional spermathecae and a central rudimentary one (Edwards, 1939).

The results obtained are given in Tables 4 and 5 28 of the 30 impunctatus females examined had been fertilised. These included blood-fed and unfed specimens, some caught flying and others which had entered a henhouse to feed. Collections were made both in the morning and in the evening. With heliophilus on the other hand only one

out of the fifteen females dissected had been fertilised.

The high percentage of fertilised females of C. impunctatus obtained would seem to indicate that mating takes place either directly on emergence or at least before flying and feeding behaviour begins. If this is so it would not be necessary for males to fly long distances from the breeding sites, and this might in part account for the peculiar sex-ratios obtained by various workers.

The results obtained with C. impunctatus resemble those obtained for C. austeni by Nicholas (1953). He found that 11 of the 12 specimens he dissected had spermatozoa in their spermathecae. In the laboratory however, biting was not inhibited by the lack of mating. The fact that one of the two female C. impunctatus which had not been fertilised was blood-fed shows that in this species also mating is not essential to feeding. This is also the case with most mosquito species, although higher biting rates for fertilised females than for unfertilised were recorded by Bates for Anopheles atroparvus and by Burgess and Young for A. quadrimaculatus (quoted by Bates, 1949).

C. heliophilus must differ in its mating habits from C. impunctatus and C. austeni, since only one of the specimens had been fertilised. Here also two specimens which

had not been fertilised had nevertheless taken a blood-meal.

Summary.

1. The stages of development of the ovum of C. impunctatus were described and assigned to five stages. The gonotrophic cycle of C. heliophilus was also studied and found to be similar.

2. C. impunctatus could not be induced to develop beyond Stage 2 of the gonotrophic cycle without a blood-meal. C. circumscriptus was also found to be anautogenous. C. riethi was however, shown to be able to develop ova to maturity without taking a blood-meal.

3. The aerial population of C. impunctatus was almost entirely made up of unfed females with ovaries at Stages 1 or 2 of the gonotrophic cycle.

4. An unsuccessful attempt to suck resting midges out of herbage with a trap devised by Johnson and others (1955), is described.

5. Ampulla diameter measurements made on the oviducts of wild-caught C. impunctatus showed a bimodal distribution, thought to correspond with the nulliparous and multiparous sections of the population.

6. 28 out of 30 female impunctatus dissected had been

fertilised, while only 1 out of 15 heliophilus females had spermatozoa in their spermathecae. It was therefore thought likely that C. impunctatus mates very soon after emergence, whereas C. heliophilus undertakes some other aspect of its behaviour first.

-o-o-

# PART II

## MATERIALS AND METHODS.

Campbell and Pelham-Clinton (personal communication) have listed the species of Culicoides taken at Lephinmore. Of these only C. impunctatus and C. heliophilus occur in sufficient numbers to make statistical analysis of the results possible. C. heliophilus is most abundant at the end of June and the beginning of July (Parker 1949), but after the end of July it appears only rarely in the catches. C. impunctatus is available in appreciable numbers well into September, and thus provides a considerably longer working season. This species only was used in the investigations on the relations between climatic factors and abundance.

Collections were made with a type of suction-trap devised by Johnson (Johnson 1950 a and b, Taylor 1951). A Vent-Axia fan sucked flying insects into a metal-gauze cone and thence into a collection-tube. A pile of metal discs was supported below the fan-motor by the two jaws of a release mechanism. This was operated by a time-switch which at preset intervals allowed current to pass through a solenoid which became magnetic and pulled the release to one side. This allowed the last disc to fall down a

central rod into the collecting-tube, while the penultimate one replaced it on the lower of the two shelves of the release. Londex time-switches were used instead of Venner, which were used in the prototypes (Johnson 1950 a and b, Taylor 1951) at Dr. Johnson's suggestion. Throughout the trapping the time-switch was set so that discs were dropped at hourly intervals, so that the catch was sorted automatically into hourly samples. The collecting-tubes were changed at 24-hourly intervals. It was found most convenient to do this at mid-day when activity was low, so that the presence of the experimenter, providing a source of blood, caused as little disturbance as possible in the normal pattern of activity.

The discs were fringed with rubberized cloth instead of Egyptian cotton, since Campbell and Pelham-Clinton (personal communication) found that this material stood up to prolonged soaking better under field conditions. They were coated with an acetone extract of pyrethrum, which gave a quick knock-down to the insects caught but did not kill them. They were kept therefore, before the tubes were opened, for one hour in a closed container with some ethyl acetate soaked on cottonwool in order to kill the insects. On very wet days it was necessary to dry off the catch with gentle heat before attempting to remove specimens

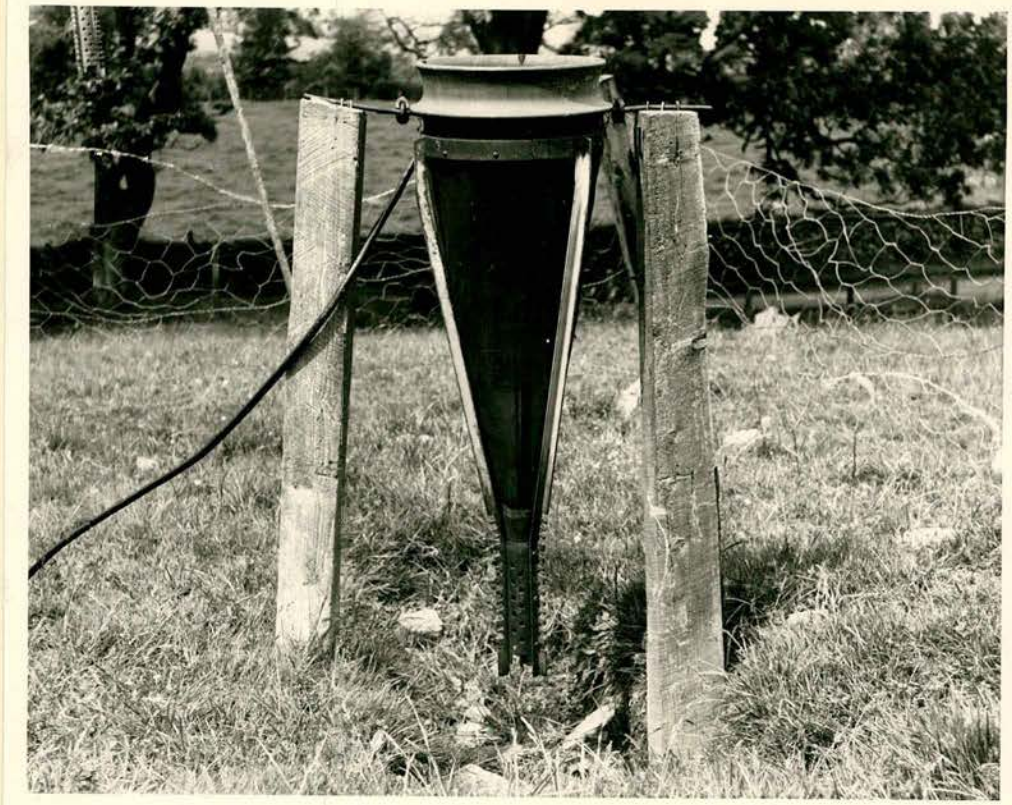


Figure 8. Trap B in situ.

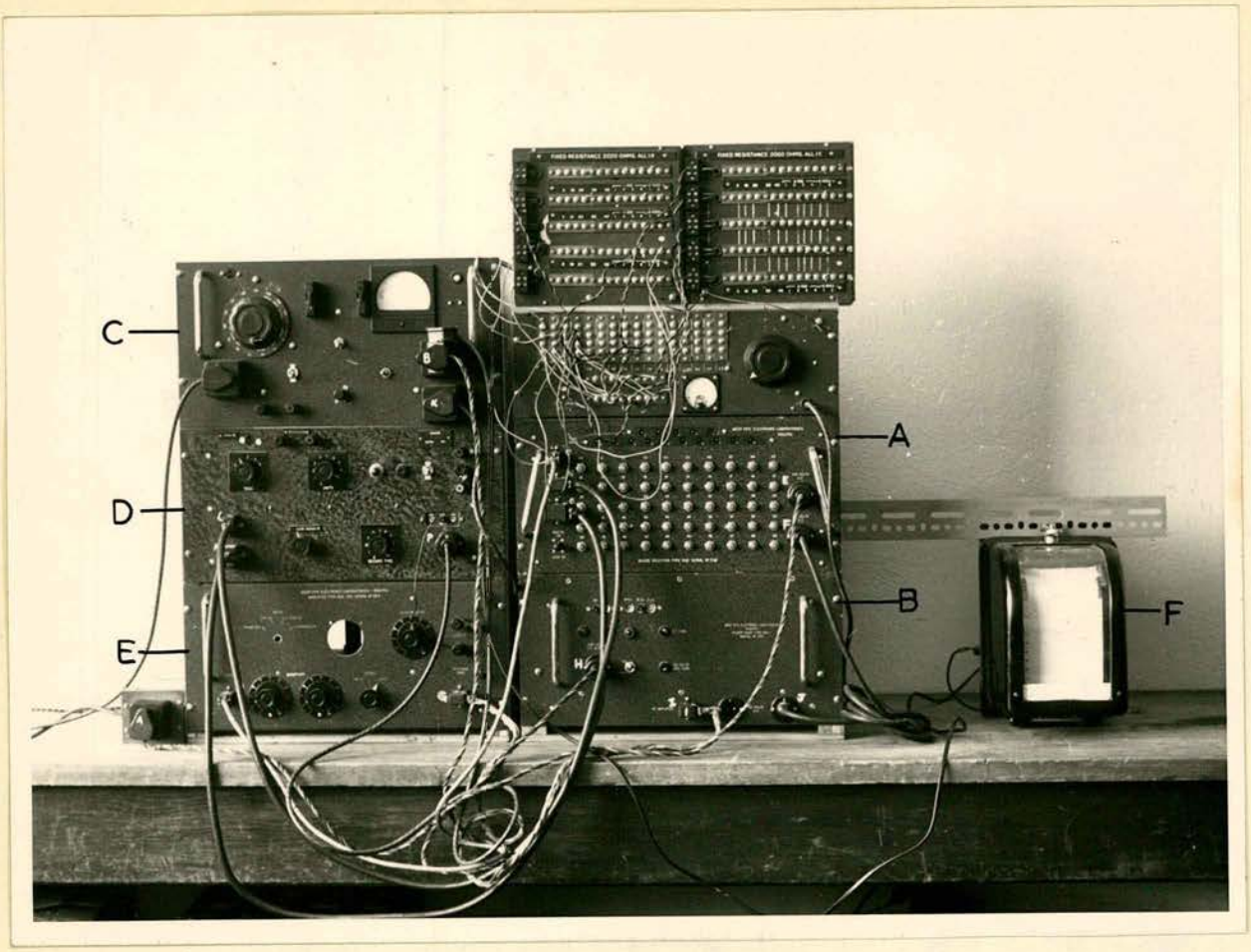
from the discs. It was generally possible to get most of the catch off in a recognisable state. The rest were recorded as unidentifiable.

Three of the traps were operated simultaneously within the enclosure. These are referred to throughout as Traps A, B and C; they had their mouths at 6 ft., 3 ft., and 6 in. respectively above the ground. The slope of the ground within the enclosure was approximately 1 in 2.3. On the assumption that height above the ground is more important to a flying insect than actual height above sealevel, no correction for slope was made.

The 6 in. trap was suspended by its rim from a hole in a sheet of fibre-board laid over a pit dug in the ground. Turves were placed over the fibre-board to give this trap when seen from above, a similar background to traps A and B. Trap C was lifted up and supported on a pair of posts while the collection-tube was being changed. After heavy rain the level of soil-water rose and the pit was sometimes flooded. When this occurred the trap was switched off and removed from the pit to dry out. This accounts for the discontinuity of the records for trap C.

Meteorological Conditions.

Simultaneously with trapping, recordings of climatic


C
D
E
A
B
F

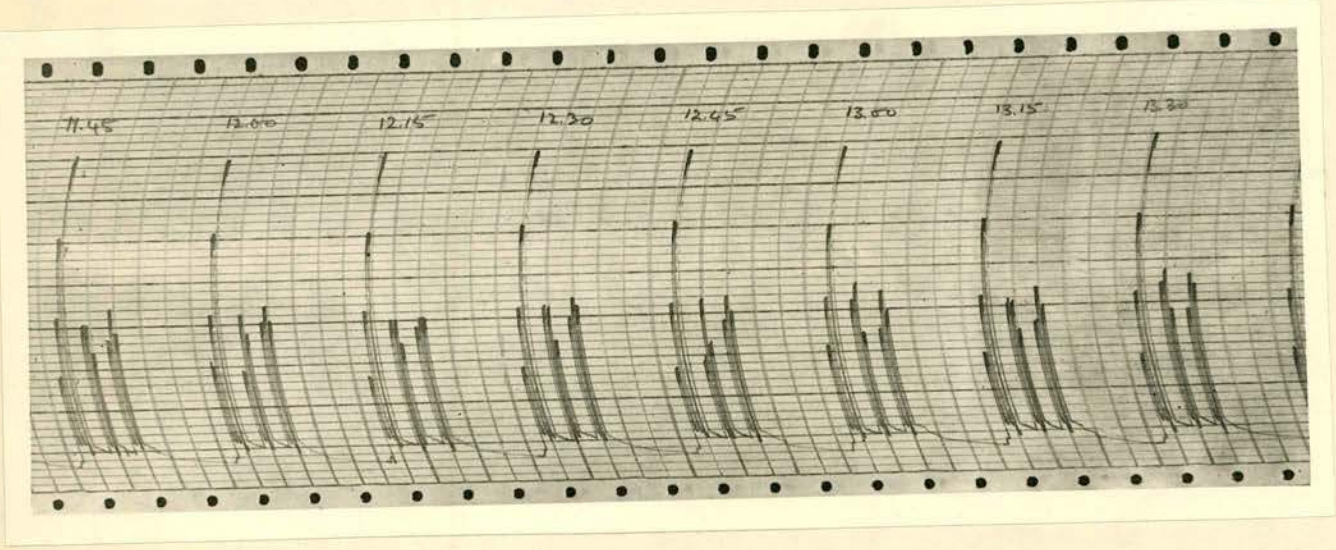

11.45
13.00
12.15
12.30
12.45
13.00
13.15
13.30

Figure 10

The electronic switch and recorder used for recording temperatures at three heights.

A = unit containing Wheatstone Bridge Systems.

B = Amplifier. C = Power unit.

D = Sequence control unit.

E = Phase sensitive detector. F = Recorder.

Figure 11

Type of record obtained.

conditions were made. Only one recorder was available, an Everett Edgecumbe Inkwell Graphic Ammeter. The problem of recording a number of different impulses with a single pen was resolved by using an electronic switch to select each in turn in a predetermined sequence. This apparatus was designed by the West Fife Electronics Laboratories, and the following is virtually the makers' description:

> The equipment comprised a remote-controlled AC excited Wheatstone Bridge system for use with various transducers such as Thermistors, Pressure Gauges etc. There were two independent 25 Channel Bridges with variac controlled bridge supplies, which could be set to any voltage from 0-6 volts 50 c.p.s. Channel selection was accomplished either by a panel switch, or remotely, and the channel selected was indicated by lights on the panel. The remote operation of the channel selecting switch was performed by a Sequence Control Unit, which, after the closing of a pair of remote contacts (on a time-switch), would select each channel in turn, and allow a predetermined record period followed by a similar period during which the recording meter was disconnected; thus giving a base line after the record for each channel. Switching was provided so that when the last channel in use had been monitored, the whole

equipment was reset and awaited the next signal from the time-switch.

The output of the selected bridge channel was fed into an amplifier. The amplifier output was fed into the phase sensitive detector which was designed to activate the 0-1 ma. recorder of internal resistance 1000 ohms.

All power to control the channel selection, indication and the various control relays was provided by a power unit supplying 50 volts D.C. This power unit also provided the supply for the amplifier.

Figure 10 is a photograph of the apparatus and the ammeter.

Not all the 50 possible channels were used. Four fixed resistance ratios and nine thermistors were decided on, but for reasons explained later, three of the thermistors were not used after the preliminary experiments. It was found that with a chart-speed of 6 in. per hour and a record time of 12 secs. it was possible to get clear separation between successive readings. The time-switch was set so as to complete the circuit every quarter of an hour, so that there was a complete set of readings every fifteen minutes.

The four fixed resistance ratios were put in as calibration steps. The mains voltage was found to be

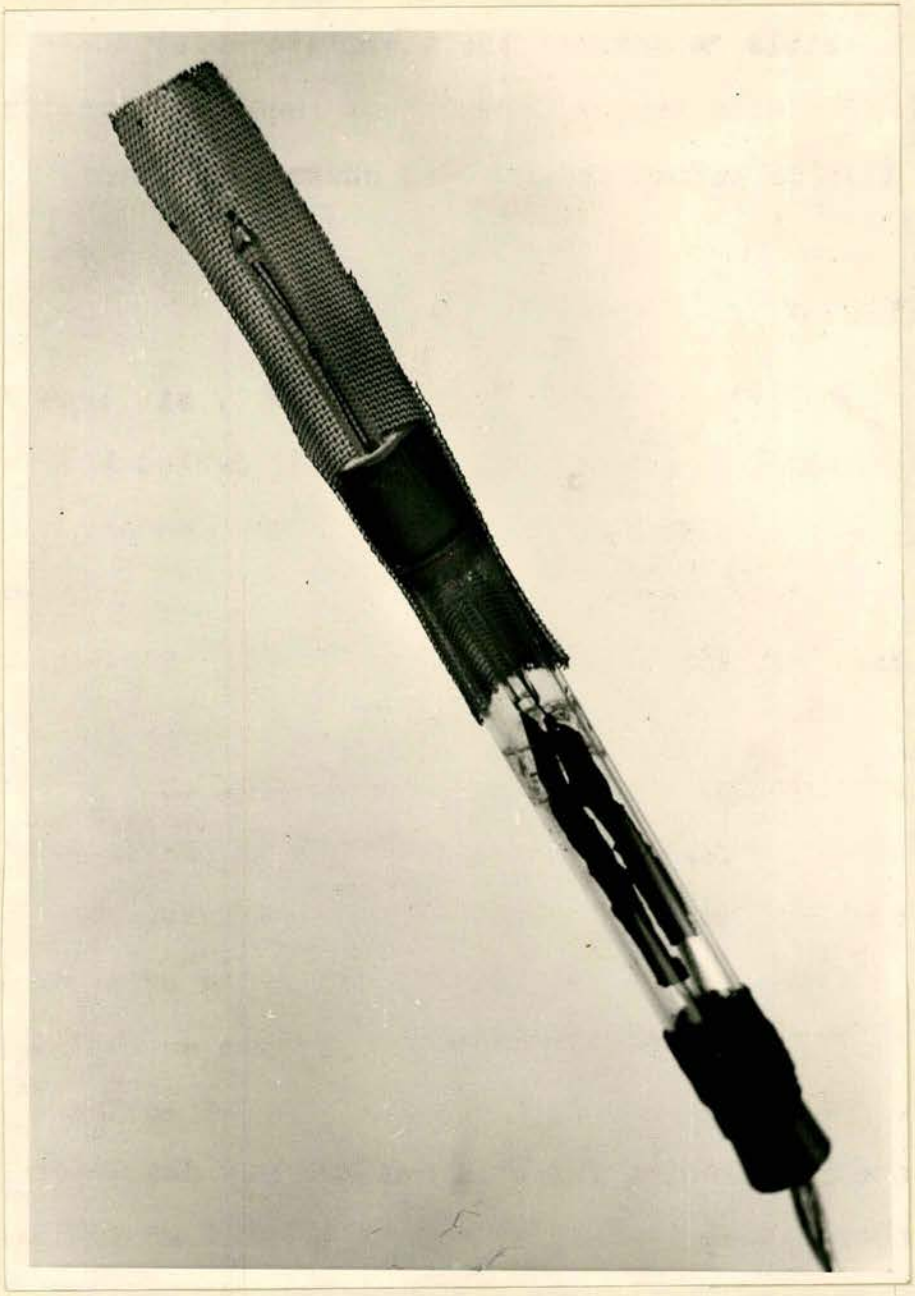


Figure 12. Thermistor, with radiation shield.

variable, there being an increase during the night. It was possible to correct the thermistor-resistance readings with reference to the position of these calibration steps. The type of record obtained is shown in figure 11.

Temperature and Humidity.

Following Penman and Long (1949), six type F thermistors, made by Standard Telephones and Cables Ltd. were used. They were arranged in pairs to give wet and dry bulb readings at 6 ft., 3 ft., and 6 in. above the ground, corresponding with the three trapping levels. No lagging was used. White-painted hemicylindrical shields of metal gauze protected them from radiation from above. (See fig. 12.) Tubular wicks, supplied by the Meteorological Office, were used for the wet bulb thermistors. These gave a more uniform wetting than the more usual sleeve type.

Balancing resistances were chosen so that temperatures from $0^{\circ}$ to $40^{\circ}$ C. were accommodated on the width of the chart, allowing for drift either way due to change of voltage. Each thermistor was calibrated in arbitrary units related to the divisions on the chart, instead of in ohms.

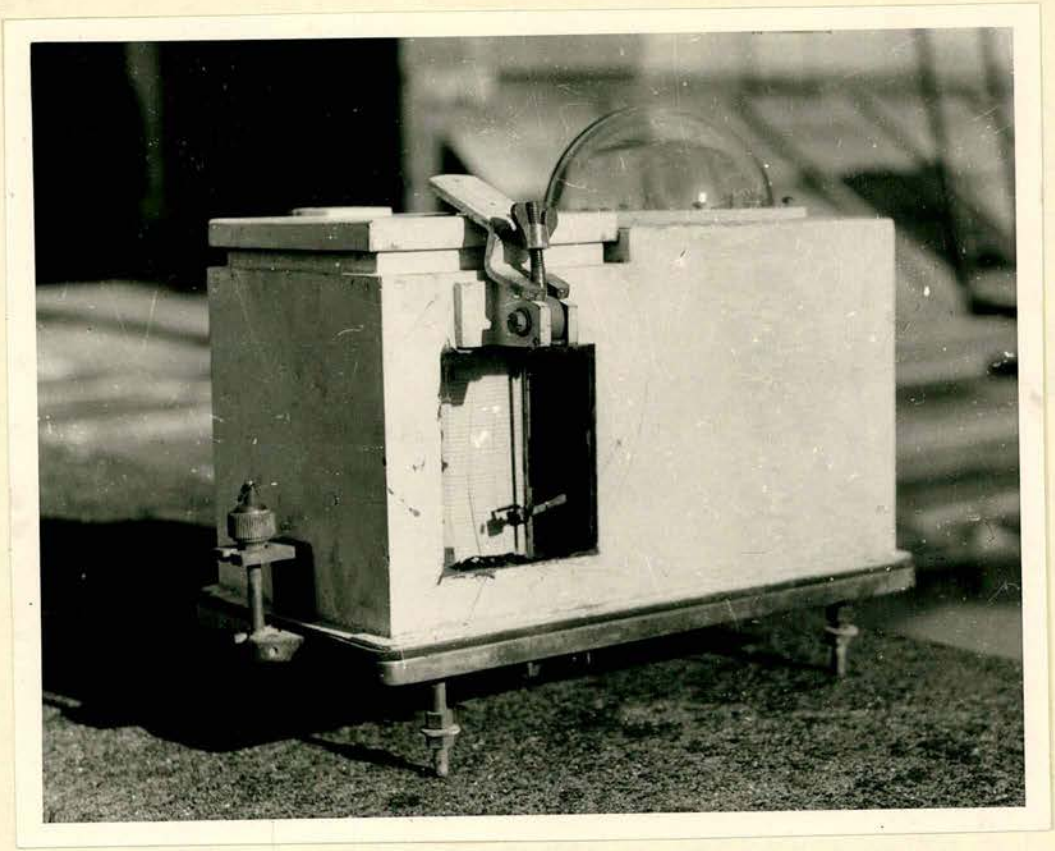


Figure 13. The radiation recorder.

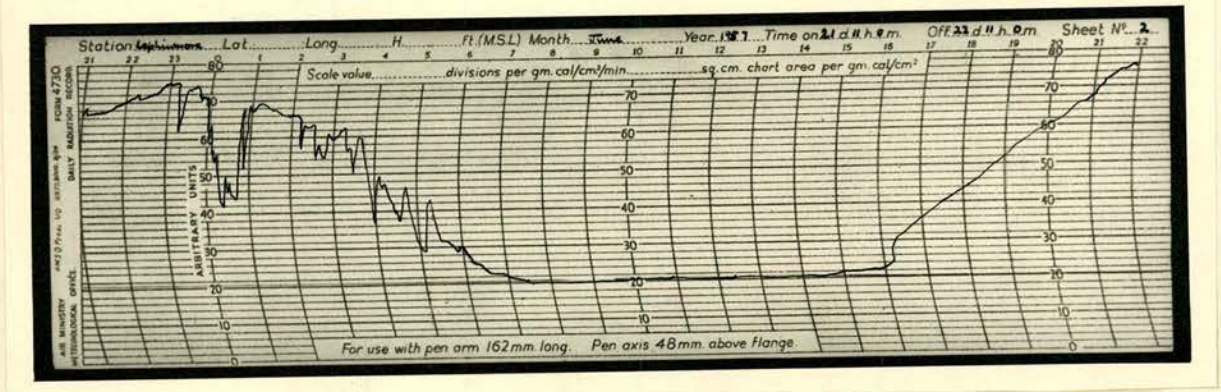



Figure 14. Type of record obtained.

Wind.

Again following Penman and Long (1949), type L thermistors were to have been used for the measurement of wind speed at the three heights. It was found however that the instrument was not suitable for continuous recording since moisture condensed on the exposed bead. After the preliminary trials therefore, they were abandoned. The daily run of wind was recorded at the meteorological station at the farm, with a cup-anemometer, and these readings were used to correlate wind with daily midge activity.

Radiation.

It is not possible to incorporate a photo-cell into an electronic circuit. A self-contained recording instrument was therefore required. The funds available were not sufficient to purchase a light recorder. The idea of measuring light intensity was therefore abandoned. A radiation-recorder designed by Blackwell, and made commercially by Cassella and Co. was borrowed from Eskdalemuir Observatory. A blackened metallic strip under a glass dome caused, by its expansion, the deflection of a pen arm. The pen marked a chart driven by a clockwork

mechanism. At night the radiation was zero, and the pen dropped to a steady position, thus providing a base-line against which the amount of radiation could be measured. During the day the radiation over a period was proportional to the area between the trace for that period and the base-line. Figure 14 shows the type of record obtained.

Arrangement of Instruments.

The recorder and electronic switch were housed in a wooden hut just outside and to the north of the enclosure within which the three traps were placed. There were leads from the hut to the thermistors, which were attached to a post 10 ft. within the enclosure.

The radiation recorder occupied the south-west corner of the enclosure. In this position it was not in the shadow of any of the other instruments at any time of day.

The traps occupied the eastern part of the enclosure. They were arranged more or less at the angles of an equilateral triangle of side 8 ft.. B and C were approximately at the same level on the slope, while A was lower and to the north of them.

Mathematical Treatment of the Data.

The suction trap records consisted of numbers of

midges caught per hour at the three different heights. Hourly catches were used in the comparison of activity with radiation, and with temperature and humidity. For other studies the catch for a period of 24 hours was taken as the unit. The period selected was from mid-day to the following mid-day. Since there is a natural cessation of activity in the middle of the day it seems reasonable to consider morning activity as being a continuation of that begun on the previous evening, and to treat the two together as a unity. A consideration of activity from midnight to the following midnight would give an artificial grouping. Accordingly, except where otherwise stated, "daily activity" means activity for the period beginning at 12.00 on a given day to 12.00 on the next day.

The main statistical methods used were Analysis of Variance, Correlation and Linear Regression (Snedecor, 1937). The Multiple Regressions performed on the data for temperature, humidity and activity were calculated according to the method described by Woolf (1951). Curve-fitting to the data on seasonal distribution of the catch was done with the assistance of Dr. L.J.Hale of this Department.

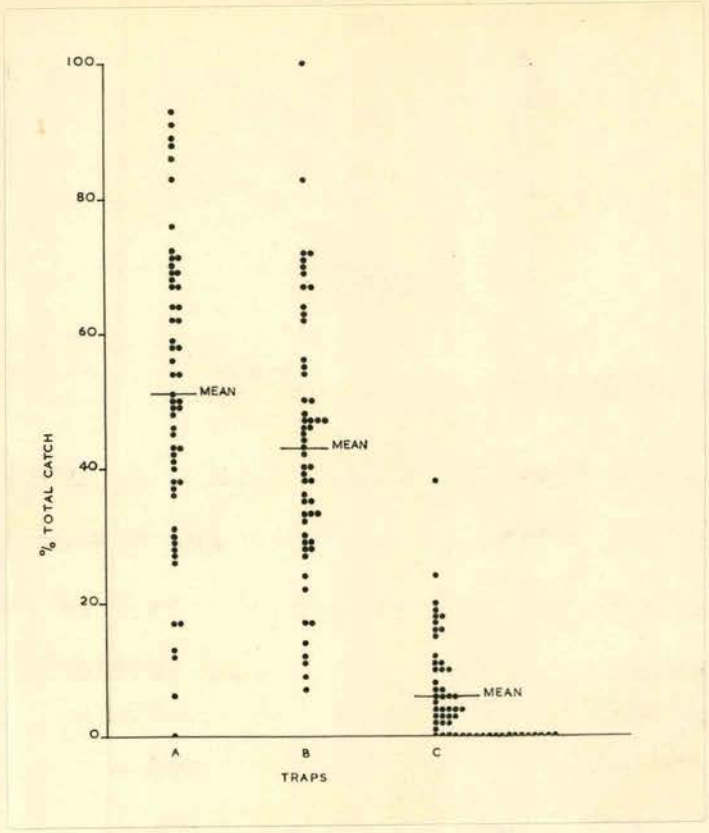



Figure 15. The vertical distribution of C. impunctatus in 1957.

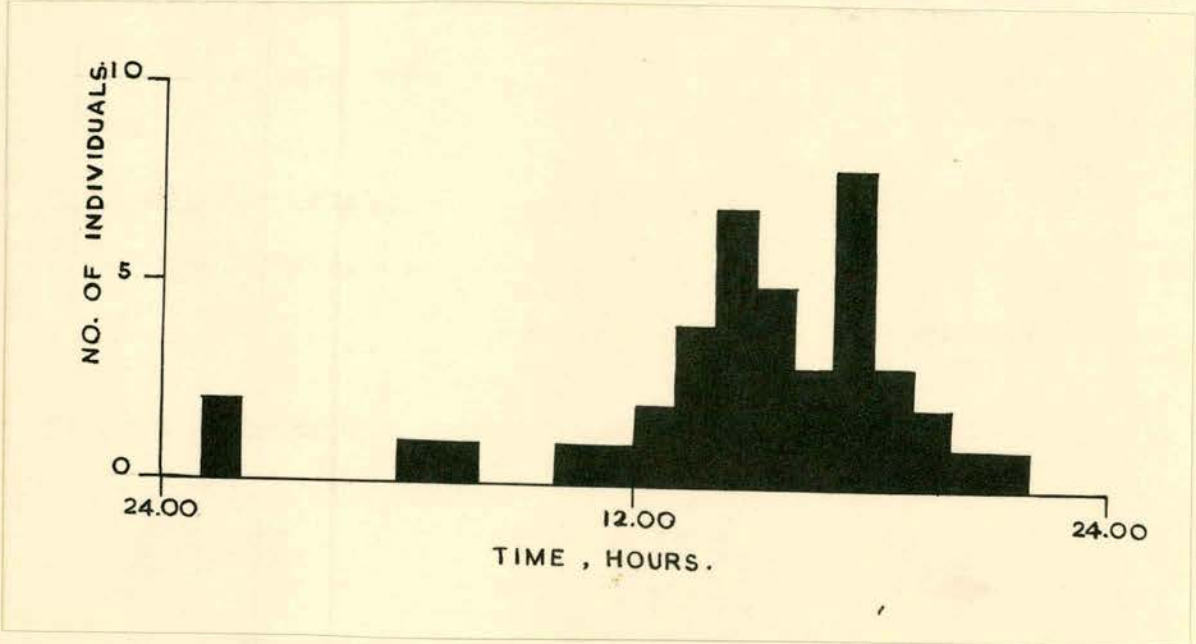



Figure 16. Numbers of Haematopota spp. caught at different times of day in 1957.

## RESULTS

### Vertical Distribution of the Catch.

A grand total of 27,587 female C. impunctatus was caught over the whole of the trapping season in 1957. These were distributed between the three traps as follows:

| Trap | Total Number | Number of days | Arithmetic mean | Mean % |
|---|---|---|---|---|
| A | 13,867 | 71 | 195.2 | 50.9 ± 6.1 |
| B | 12,260 | 71 | 172.7 | 43.0 ± 5.4 |
| C | 1,460 | 58 | 25.2 | 6.1 ± 2.1 |

For catches for individual days see Table 6. Mean percentages were calculated only on the basis of the fifty-five days when data was available for all three traps and the total catch for the day exceeded ten. The distribution of the catch between the traps is shown graphically in Figure 15.

The results obtained may be compared with Kettle's (1951) results on the vertical distribution of the females of this species on moorland:

| Height | Wester Bannachra moorland | Bannachra moorland |
|---|---|---|
| 10 ft. | 36.2 % ± 2.2 | 40.5 % ± 3.5 |
| 6 ft. | 34.2 % ± 1.9 | 38.5 % ± 3.3 |
| 2 ft. | 29.6 % ± 2.2 | 21.0 % ± 2.7 |

There would appear to be no significant difference between Traps A and B, but C caught significantly fewer midges throughout the season. C. impunctatus were not therefore flying in large numbers near the ground. There was a more or less even distribution between the three and six foot levels, and Kettle's results seem to indicate that there is no fall off in numbers between 6 ft. and 10 ft. His figures show a distinct falling off at 2 ft. however.

Work on the vertical distribution of other species of Culicoides has also been done. Snow (1955) stated that five species, C. guttipennis, haematopotus, paraensis, spinosus, and borinqueni, have a general canopy preference as shown by biting activity at 30 ft., while C. travisi is more active at shrub level. Lumsden (personal communication) using suction-traps in Uganda found that a trap at 17 metres caught more Culicoides spp. than those at 21 and 7 metres. For the same trapping period the numbers he obtained were

| Height of trap (metres) | Catch of ♂ & ♀ Culicoides spp. |
|---|---|
| 21 | 189 |
| 17 | 619 |
| 7 | 2 |

Neither of these workers, however, made observations on the vertical distribution of Culicoides in open country compar-

able to the trapping site where the present work was carried out.

The clegs (Haematopota spp.) which were caught during the season showed the opposite type of distribution to C. impunctatus. All the specimens caught were females.

| Trap | H. pluvialis | H. crassicornis | % total catch |
|---|---|---|---|
| A | 3 | 0 | 7.1 |
| B | 6 | 0 | 14.3 |
| C | 32 | 1 | 78.6 |

There are three possible explanations of the larger number of clegs caught in C.

(1) There were more clegs flying at 6 ins. above the ground than at 3 ft. or 6 ft. This sort of reasoning would be tenable with weak fliers like aphids or midges, which are sucked passively into the traps, but larger and more active insects can be expected to be able to elude them. Thus the numbers caught at different heights above the ground may reflect a behaviour difference rather than a distribution pattern. Another hypothesis is that:

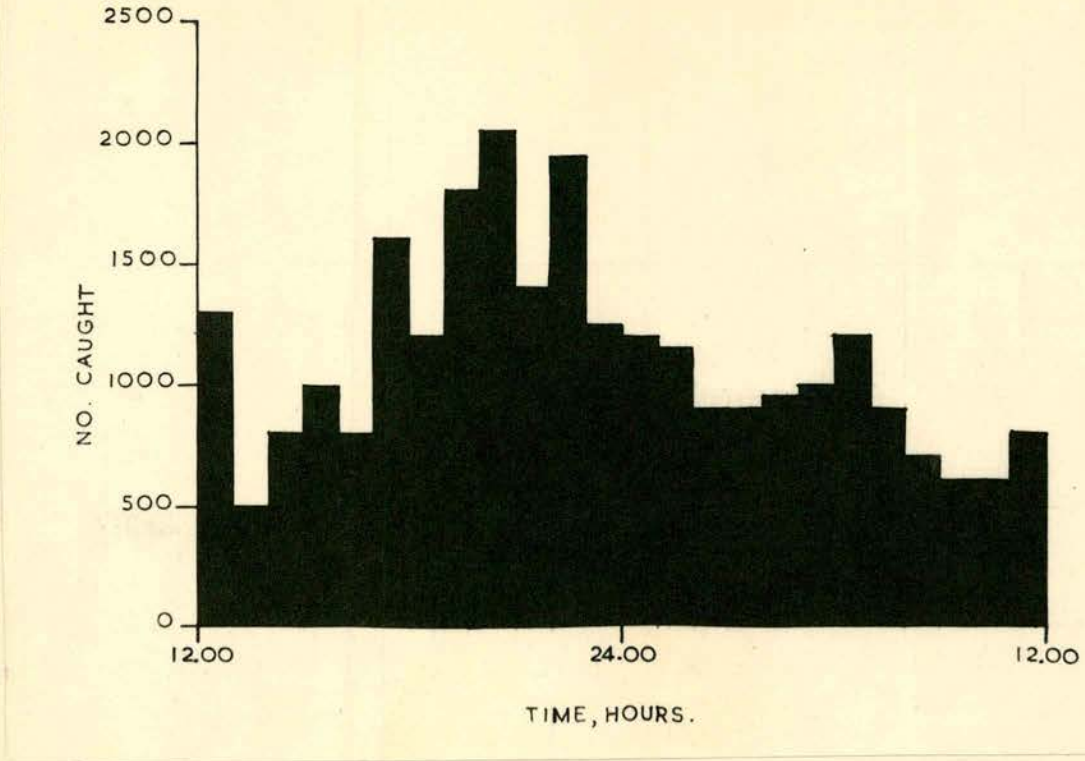
(2) The clegs were flying more slowly at 6 ins. above the ground than at the other heights and were therefore being caught in C while they flew past the mouths of A and B. Kettle (1957) has shown that clegs are active under hot, dry

and bright conditions. Under such conditions the air layer nearest the ground has a higher temperature than the layers above it (Geiger, 1950; Best et al, 1950; Robinson and Rider, 1950). By simple orthokinesis animals may show a higher rate of movement under less favourable conditions (Fraenkel and Gunn, 1940). Thus the clegs may be flying more slowly in the air layer where conditions are most favourable. Figure 16 shows the hourly distribution of the clegs caught. It will be seen that most were caught in the afternoon, the largest number coming in between 17.00 and 18.00 hours.

(3) The possibility that the clegs were being caught as they were coming down to rest on the ground cannot be ruled out.

Hourly Variation

Well defined activity cycles are known for several species of Culicoides. Nicholas (1953) found that C. austeni bites all night while C. grahamii shows morning and evening peaks of activity. Parker (1949) caught maximum numbers of C. impunctatus in the evening, at a time related to the time of sunset. There was a smaller rise in activity at sunrise, and they were almost absent at other times. These findings were not entirely borne out in the present investigations. The numbers of C. impunctatus caught at


2500
2000
1500
1000
500
0
NO. CAUGHT
12.00
24.00
12.00
TIME, HOURS.

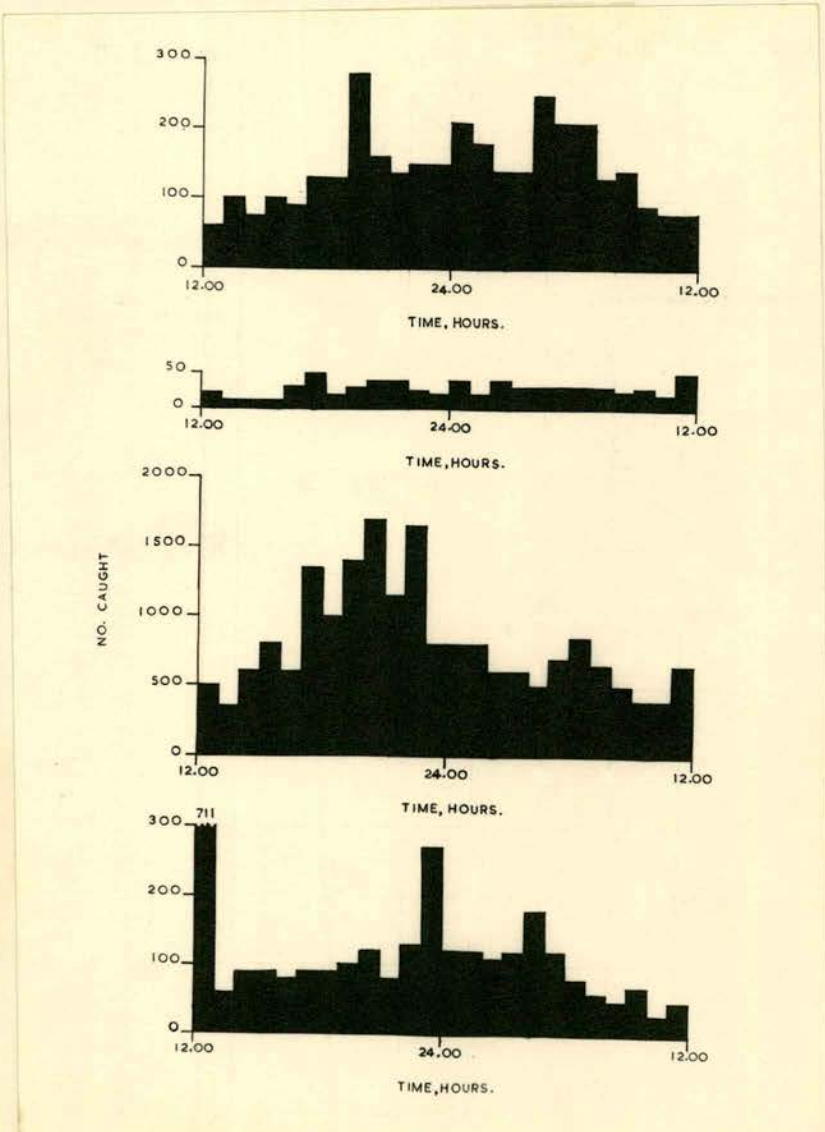

300
200
100
0
12.00
24.00
12.00
TIME, HOURS.
50
0
12.00
24.00
12.00
TIME, HOURS.
2000
1500
1000
500
0
NO. CAUGHT
12.00
24.00
12.00
TIME, HOURS.
711
300
200
100
0
12.00
24.00
12.00
TIME, HOURS.

Figure 17

Hourly distribution of the catch of C. impunctatus over the trapping season 20. 6 - 21. 9. 1957.

Figure 18

Hourly distribution of the catch of C. impunctatus over the periods:

20. 6 - 14. 7

18. 7 - 2. 8

2. 8 - 20. 8

3. 9 - 21. 9. 1957, respectively.

different times of day, totalled over the whole of the season, are given in Table 7. Figure 17 is a histogram showing this distribution. It is clear that the level of activity at night remains high, as was stated by Edwards (1939), maximum activity occurring before midnight.

For more detailed analysis the season was broken up into four periods, and the total catches for each hour within these periods are given in Table 7. Trapping was not continuous over the season and the four periods were selected so that each included roughly the same number of trapping days. Figure 18 shows the pattern of activity within each of these four periods. Before June 21st the time of sunset grows gradually later each day, and after that date shifts gradually forward again. On comparing the hourly distributions of the catch shown in figure 18 it is apparent that there was no corresponding change in the relative position of maximum activity. The lack of definition of the evening peak of activity cannot therefore be explained merely by the fact that the time of sunset varied over the season.

An analysis of variance was performed on the figures in Table 7.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 95 | 14,187,806.63 | |
| Periods | 3 | 9,283,929.38 | 3,094,643.13 |
| Hours | 23 | 1,003,242.38 | 43,619.23 |
| Discrepance | 69 | 3,900,634.87 | 56,530.94 |

The Between-hours variance was found to be less than Discrepance, and therefore insignificant, while there was a large and significant difference between periods, i.e. seasonal variance.

An analysis of variance was also performed on the logarithms of the totals given in Table 7. The effect of the transformation into logs. is to minimise the effect on the mean of unusually large or unusually small numbers, which weight it unduly when arithmetic means are being used. The results were as follows.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 95 | 28.73 | |
| Periods | 3 | 24.73 | 8.243 |
| Hours | 23 | 1.57 | 0.068 |
| Discrepance | 69 | 2.43 | 0.035 |

A Variance Ratio (F) of 1.94 was obtained between hours and discrepance which for 23 and 69 degrees of freedom corresponds to the .05 level of probability.

It was therefore concluded that although there might be a significant difference between the hours of the day as regards activity, the diurnal cycle, if it existed at all, was not as well defined as was described by Parker (1949). There was a generalised increase in activity in the evening, but the basic pattern was greatly modified by other factors,

the effects of which are considered later.

Seasonal Distribution of the Catch.

The seasonal cycle of C. impunctatus has been dealt with by several authors. Hill in 1947 stated that she first took adults in late April, that there was an increase in numbers throughout May, a peak in June and afterwards a steady decline, and that the season came to an end in August. Cameron (1948) obtained maximum emergence from the last week of May to the first week of June, and did not take any after August. Parker (1949) found maximum numbers flying in June and early July, and suggested that the difference between his results and those of Hill were due to the later emergence of adults the further north one went. None of these authors appear to have noticed any falling off in numbers during the season and subsequent recovery. In 1950 Kettle showed that the seasonal distribution of C. impunctatus as sampled by sticky traps was bimodal in 1947, 1948 and 1949, with peaks in June and July. The hypothesis that unfavourable weather conditions were responsible for the small numbers caught in the middle of the season did not adequately explain these results, and it was concluded that the bimodal distribution was the normal pattern of emergence for this species. Kettle sug-

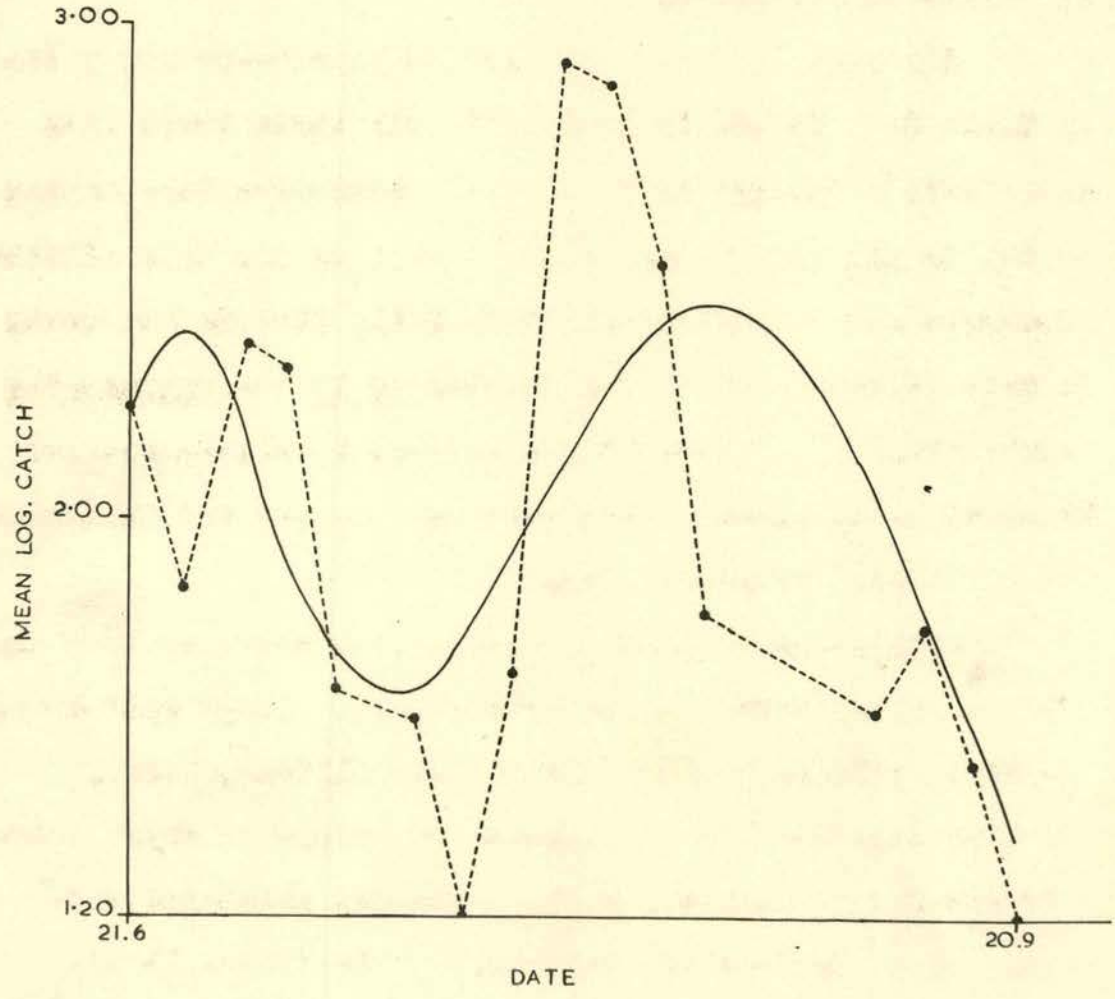



Figure 19. The seasonal distribution of the catch of C. impunctatus in 1957.

Broken line: mean catch obtained.
Continuous line: theoretical curve fitted to the data.

gested that this might be due to the fact that the adult population was made up of two biological races with different times of emergence.

The daily catches for 1957 at Lephinmore are given in Table 8. The daily totals for all three traps have been used. The variation between successive days cannot be due to population changes, and must be due to a climate dependent activity factor. This daily fluctuation tends to mask seasonal trends due to changes in the impunctatus population. In figure 19 the catches have been grouped in order to bring out these seasonal changes and eliminate minor changes due to weather.

Logarithms of actual numbers were used, so that the effects of abnormally small or abnormally large catches on the mean would be minimal (following Williams, 1937). The days were grouped in five-day periods, but where trapping was discontinuous four or three-day groupings were used. (See Table 8 for details.) In figure 19 the mean for each group has been plotted against the mid-date for the period. A bimodal distribution emerges, similar to those obtained by Kettle (1950).

A smooth theoretical curve was fitted to the data (fig. 19). The curve finally selected was a cosine curve

of increasing amplitude. This was fitted in the following way. A straight line regression was first calculated from the data, using the formula: $y = a + bx$. Then a curve having the form, $Y = - px \cos \lambda x + q$, was fitted to the divergences from the straight line regression (Y), where p = the amplitude, $\lambda$ = the wave-length, and q = a correction for the origin. The calculated values of Y were used in calculating regression in a test of significance: -

| Item | Sum of Squares | D. f. | Variance |
|---|---|---|---|
| Regression | 1.4337 | 1 | 1.4337 |
| Remainder | 2.4708 | 13 | 0.1901 |
| Total | 3.9045 | 14 | |

t = 2.746

For 13 degrees of freedom this value corresponds to a probability of .02 . The curve was therefore considered to be an adequate fit.

Curve fitting was undertaken on the assumption that the normal pattern of emergence is bimodal, and that the curve would represent population change over the season. One could then try to explain the difference between calculated and actual figures in terms of climatic factors. In practice, however, the curve chosen, though it gave a significantly good fit, was far from ideal. The periods

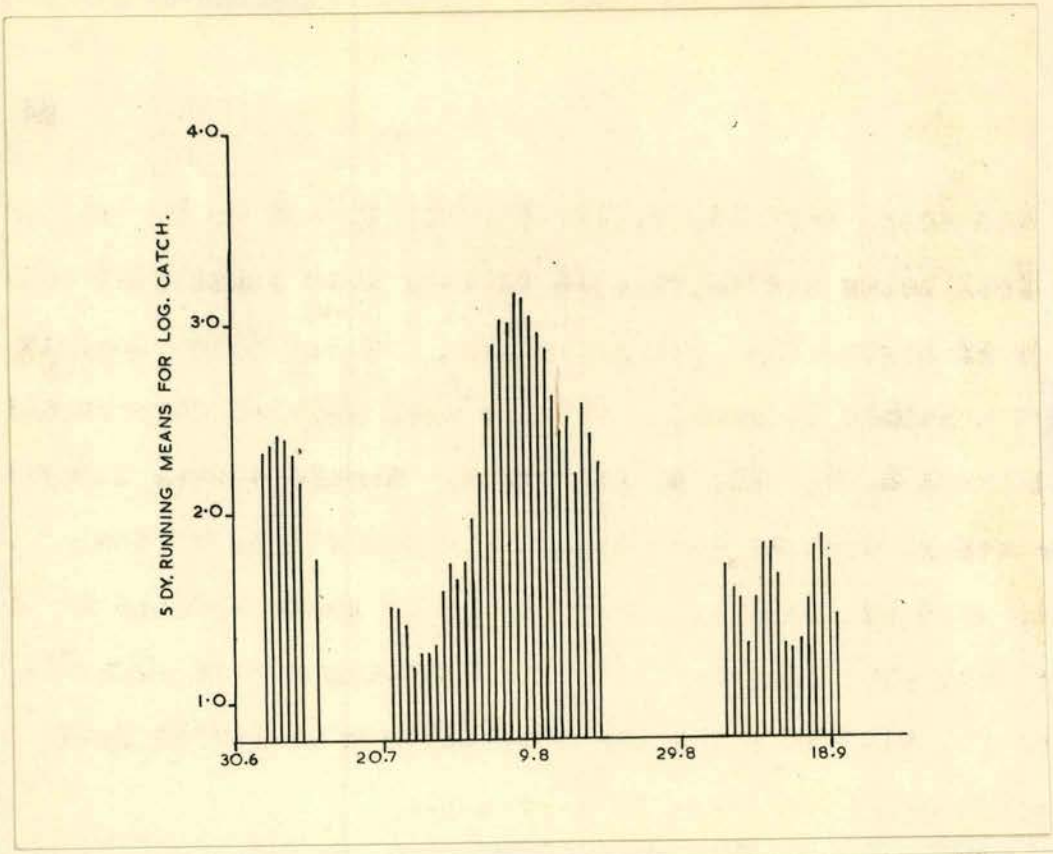



Figure 20. Seasonal distribution of C. impunctatus in 1957 as shown by 5 day running means.

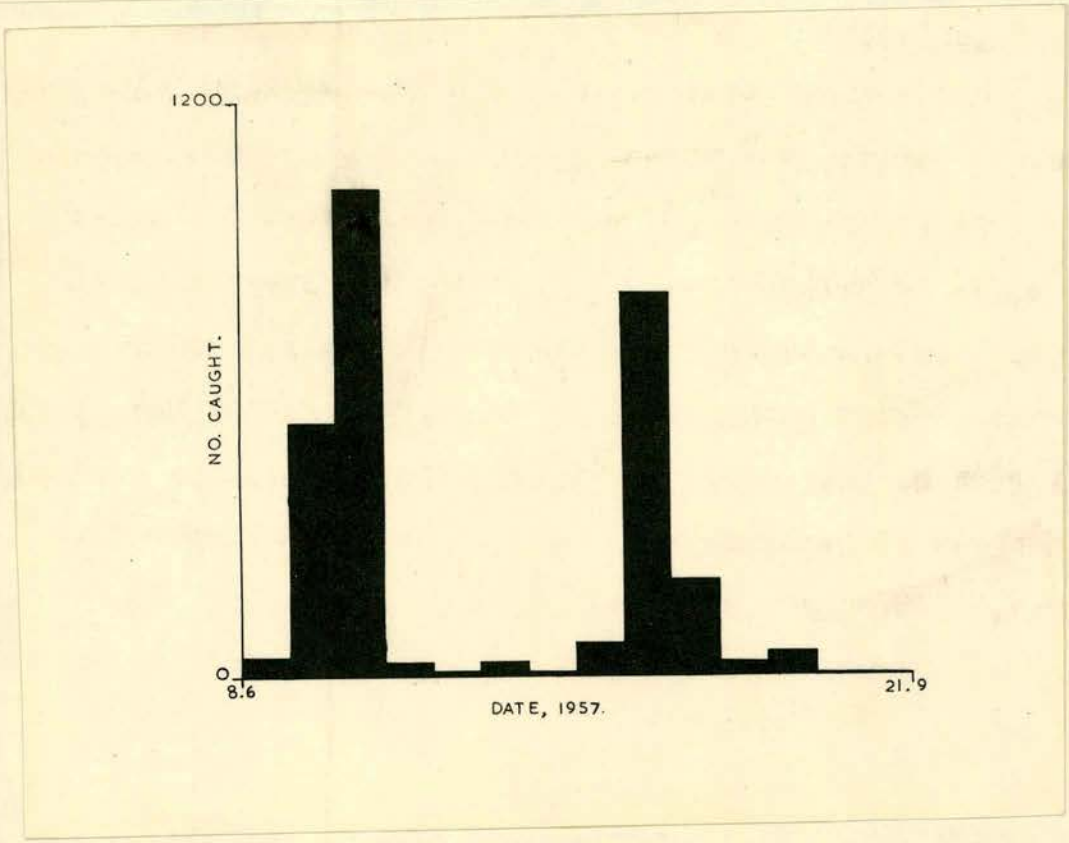



Figure 21. Seasonal distribution of C. impunctatus at Strachur in 1957. (Parish, unpublished.)

whose mid-dates were 26. 7, 19. 8 - 20. 8, and 6. 9, all of which fell below the calculated values, were associated with periods of higher than average wind. On the other hand it was not possible to explain why the mean catches corresponding to mid-dates 5. 8, 10. 8 and 15. 8, should exceed theoretical values as much as they did. A curve fitted by the methods used is therefore only partially successful as an index of population level. The fluctuations due to climatic factors are too great for the true pattern to emerge from curve-fitting on the data as it stands.

It was therefore decided not to use this curve in further work on the effect of climatic factors on the activity of C. impunctatus. Following Williams (1940) five-day-running means were taken as an indication of seasonal trends. Figure 20 shows the picture which emerges by this method.

It is interesting to compare the results obtained with those of Parish (unpublished) for the same trapping period. He was working at Strachur, ten miles further up the coast, using sticky traps. Figure 21 shows the seasonal picture he obtained. A bimodal distribution is evident, but whereas at Lephinmore the second peak was larger than the first, the reverse was the case at Strachur. The bimodal distribution was therefore a real phenomenon in 1957

for that part of the West Coast of Scotland. Other results of Parish (unpublished) indicate however that there may be a single peak of activity in some years.

Bimodal seasonal distributions are known for other insects. The dragonfly Anax imperator is an example (Corbet, 1954, 1955 and 1956). Corbet explained this time-frequency distribution as the result of the existence of two groups of larvae, the first of which entered the final instar before June, and in response to the increasing photoperiod, did not diapause. These formed the second peak of the same year. Larvae entering the last instar after June went into diapause over winter and matured in the spring of the following year, when they constituted the first peak. An alternative explanation is that of Johnson and Taylor (1957) who pointed out the similarity between such seasonal distributions and the diurnal activity pattern shown by aphids, which is also bimodal. They suggested that the causes of bimodal seasonal distributions might be similar to those responsible for double peaks in diurnal activity, which are now fairly well understood. This would mean that the rate of larval development was variable and dependent on climatic factors. Mature 4th instar larvae would accumulate in winter and pupate more or less simultaneously as soon as conditions became suitable. They would form

the first wave of emergence. This would be followed by a decline in numbers pupating, but as summer temperatures rose the rate of development would accelerate and a second peak be built up. This hypothesis would appear to fit the present case, especially as the model described is highly flexible, the size and time of the two peaks being dependent on conditions during the whole of the larval period.

The Effect of Wind on Daily Activity.

Studies by Pratt and others (1949) on the activity of Culicoides tristriatulus showed that greatest numbers were taken at wind-speeds of 1.5 to 1.9 miles per hour, counts were near or above average at speeds less than 1.9 and between 2 and 3.4 m.p.h., and there was very little activity above 3.5 m.p.h. These findings are similar to those obtained in laboratory studies on Calliphora erythrocephala by Digby (1958), where it was found that wind speeds up to 0.7 metres per sec. had an activating effect, while above this flight was inhibited. Kettle in 1957 only took specimens of C. impunctatus under calm conditions. Casual observations in the field seemed to show that there was a threshold at about 2 m.p.h. above which this species ceased to be active. It was therefore

planned to use a hot-wire anemometer which was capable of measuring accurately windspeeds below this, but for reasons which are discussed more fully in the section on Methods, this was not successful. There was a meteorological station at Lephinmore, and readings of the daily run of wind during the trapping period were availeble. These are set out together with the figures representing the total catch for the same period in Table 9. The daily run of wind, in miles of wind for 24 hours, was read at 9.00 hours. The figures for hourly activity were therefore grouped similarly into 24-hourly totals from 9.00 on one day to 9.00 on the next.

The attempt to find a correlation between the two sets of figures is open to four main sources of error.

(1) The cup-anemometer used at the meteorological station was not sensitive to wind-speeds below about 1 m.p.h.

(2) The number of miles of wind in 24 hours gives no indication of its strength and duration.

(3) Light, temperature, humidity, and time of day are other possible factors affecting activity.

(4) The seasonal effect.

Of these, (1) and (2) are inherent in the data. The effect of the other meteorological factors is discussed separately. For the seasonal effect correction is possible.

The seasonal picture is shown in figures 19 and 20 Daily totals for all three traps were compared with the daily run of wind. Actual figures could not be used in such a comparison. For instance on 2nd August there was a catch of 88, which represented low midge activity, since the population was high, whereas a total of 66 on 9th September when the population was falling, was above the average for that part of the season. Following Williams (1940) 5-day running means were taken throughout the season to obtain seasonal trends, and the difference between the actual catch and the mean was expressed as a percentage of mean. This made it possible to compare catches from different parts of the season.

The use of means poses another problem. As Williams (1937) has shown, unusually small or unusually large catches influence the mean unduly. To avoid this, the logarithms of actual figures were used, so that geometric means rather than arithmetic were being taken.

When the corrected figures for activity were used, a negative correlation coefficient of - 0.384 was obtained. This exceeded the 0.01 level of significance for 49 degrees of freedom.

It is noteworthy that the three sources of error listed above, which could not be corrected for, would all

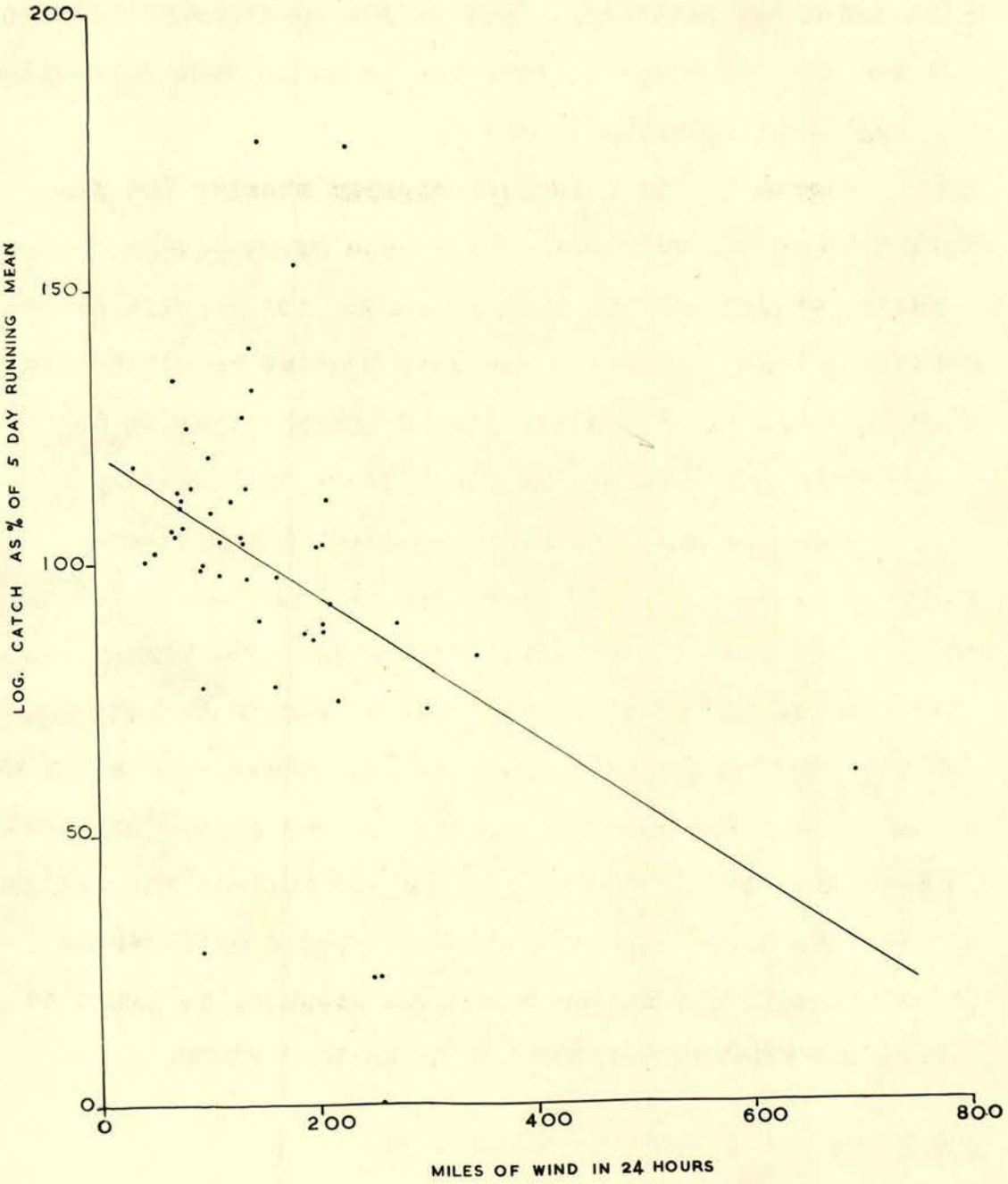



Figure 22. Scatter diagram showing the relationship of daily catch and daily run of wind.

tend to obscure any correlation that might exist between wind and midge activity. The actual correlation between the two can therefore be expected to be in reality a closer one than that actually obtained.

Figure 22 is a scatter diagram showing the distribution of the readings. The line representing the regression of activity on wind is shown, the regression coefficient being - 0.13. It must however be emphasised that this has no biological significance, owing to the limitations of the method of taking the wind readings.

When the daily catches for traps A and B were analysed separately regression coefficients of - 0.151 and - 0.157 respectively were obtained. The higher negative regressions obtained were due to the fact that when analysed separately, the catch was on several occasions all in one of the traps, while the other trap showed no catch. It was concluded from the very similar regressions obtained for the two traps that wind did not have a differential effect on activity at two different heights, at least at the higher wind-speeds dealt with in this study.

The Effect of Radiation on Activity.

Light is known to have an effect on the activity cycles of many insects. Mellanby (1940) found that the

pattern of activity of the cockroach Blatta orientalis was modified by changes in illumination. Normally active by night, the cockroaches did not emerge from their daytime resting places when a room was artificially illuminated at night. On the other hand they could not be induced to emerge simply by darkening the room during the day, unless it had been constantly illuminated the night before. Kettle (1953) showed that the biting activity of Chrysops silacea was related to the intensity of illumination during the day. Parker (1949) showed that C. impunctatus was crepuscular in its activity. Kettle's (1957) results agreed with this, increased activity being associated with low light intensities. In the present study, the effect of radiation on C. impunctatus activity was investigated, rather than the effect of light, for reasons which have been explained in the section on Materials and Methods.

Figure 14 shows the type of radiation chart obtained for a 24-hour period. Each hour was analysed separately. The radiation for an hour was proportional to the area of chart between the trace for that hour and the base-line. The mean height of the trace above the base-line for the hour, measured to the nearest millimetre, gave an estimate of the radiation for that hour. It was found

convenient to work in arbitrary units equivalent to the mean height for an hour, expressed in mm. For the ratiation-recorder used (calibrated at Eskdalemuir Observatory) a 1 mm. deflection of the pen corresponded to 1.46 gramme-calories per square centimetre per hour. Radiation-equivalents in cal./cm.$^2$/hr. for the arbitrary units used have been included in Tables 10 a and b.

The hourly catch for traps A and B was compared with radiation for that hour. Both the actual number caught, and the hourly catch expressed as a percentage of the day's catch, were used. When percentages were being used only days on which the total catch exceeded 100 were taken.

The hourly radiation readings were grouped arbitrarily into 13 classes. A class interval of 4 arbitrary units was selected, but since it was thought that low radiation intensities might be of special interest, smaller class intervals were used in grouping observations below 5 arbitrary units. In this way fluctuations due to small changes in radiation would not be masked. Accordingly Class 1 included only hours for which no radiation was recorded, Class 2 hours for which the radiation was between 0.5 a. u. and 2.0 a. u., and Class 3 between 3 a. u. and 5 a. u. The distribution of the catch between these 13

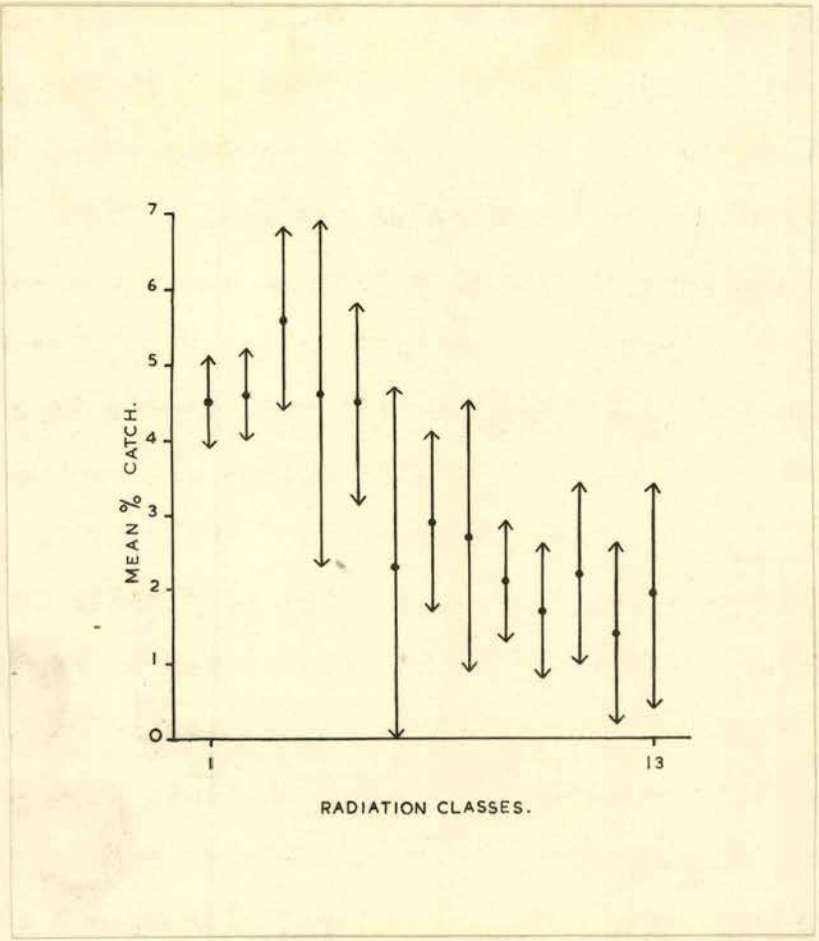



Figure 23

Distribution of the catch between thirteen radiation classes, showing means and fiducial limits.

classes, using actual figures is shown in Table 10 a, while in Table 10 b percentages have been used. Figure 23 shows the distribution of mean percentages with fiducial limits. There is a decrease in activity with increasing radiation, but the nature of the relationship between them is not immediately obvious. In the work described in the rest of this section, only percentages have been used, thus eliminating seasonal effect.

An analysis of variance between all the classes gave a Variance Ratio (F) of 2.79, which for Degrees of Freedom ($n_1$) of 12 and ($n_2$) of 679 corresponds to a probability (P) of 0.001. Thus they cannot all be regarded as belonging to the same population. Analysis of variance between classes 1 and 2 gives a value of F which is not significant. Similarly classes 1 to 3 may be regarded as belonging to the same population. Successive analyses, each including one class more than the last, show that there is no significant difference between classes until class 7 is included, when F = 2.155. For $n_1 = 6$ and $n_2 = 594$ this exceeds the 5 % point. Class 7 therefore differs significantly from classes 1 to 6.

A similar series of analyses of classes 12 and 13, 12 to 14, etc. shows values of F which are not significant till class 5 is included, when F = 2.5. For $n_1 = 8$ and

$n_2 = 218$ this corresponds to the 1 % level of probability. For individual analyses of variance see Table 10 c.

The radiation values observed therefore may be regarded as falling into two groups, one which includes classes 1 to 6, and the other classes 6 to 13. The level of activity is higher in the first group than in the second. Within each group however there are no significant differences. Thus there are two levels of activity with an intensity threshold separating them. Class 6 could be included in either group, so one would expect the threshold value to fall somewhere within this class. The threshold value therefore lies between 23.4 and 29.2 cal./cm.$^2$/hr. Radiation intensities above this value inhibit the activity of C. impunctatus, but below the threshold radiation does not appear to have much effect on activity.

If class 6 is included in the first group we have

Group I (Classes 1 to 6) Mean % = $4.53 \pm 0.44$
Group II (Classes 7 to 13) Mean % = $2.21 \pm 0.43$ .

The effect of radiation on Culicoides tristriatulus has been discussed by Pratt et al. (1949). They describe crepuscular activity for this species but attribute the fall off in numbers during the day to high winds accompanying

sunshine rather than to high radiation. With winds less than 3 m.p.h. there appeared to be a positive correlation between radiation and activity, and activity was low in total darkness. This species therefore, differs from C. impunctatus in its responses to radiation.

The light component of the total radiation varies with the angle of the sun, and with the atmospheric conditions (Blackwell, 1954). It is not possible therefore to draw any direct conclusions as to whether light intensity or total radiation is effecting midge activity. Certain inferences may, however, be drawn about the influence of light on activity. Parker (1949) found a relationship between the time of sunset and sunrise and maximum activity. He interpreted this in terms of the stimulatory effect of low light intensities. If this interpretation were correct, one would expect a significant falling off of activity during hours of total darkness. These are all included in class 1 in the present work, and as the analysis of variance has shown, this does not differ significantly from the rest of Group 1, which includes several low light intensities. Any relationship with light which exists must, like that demonstrated for total radiation, be a threshold one, below which activity can take place subject to the influence of other factors.

The Effect of Temperature and Humidity on Activity.

Kettle's preliminary study on midge activity, published in 1957, did not show any definite relationship between temperature and humidity and the numbers of C. impunctatus caught. Over 200 specimens were taken in a single observation period on two occasions, when the readings for these two climatic factors were 70° F. and 69 % R.H. and 64.5° F. and 71 % R.H. respectively. Pratt et al. (1949), working with C. tristriatulus, found that maximum activity occurred between 50° and 59° F., but attributed the fall off in the numbers caught at temperatures above this to the consistently high winds in the middle of the day rather than to high temperatures, thus suggesting that a positive correlation existed between the activity of this species and temperature. Similarly, high catches were associated with relative humidities ranging from 90 to 94 %, but high winds could again have been responsible for the fall off in numbers caught during the day.

Hourly records of midge activity were available for the 6 ft. and 3 ft. levels all through the season in 1957. For part of the time temperature and humidity were recorded at these levels. Readings were taken at 15 minute intervals,

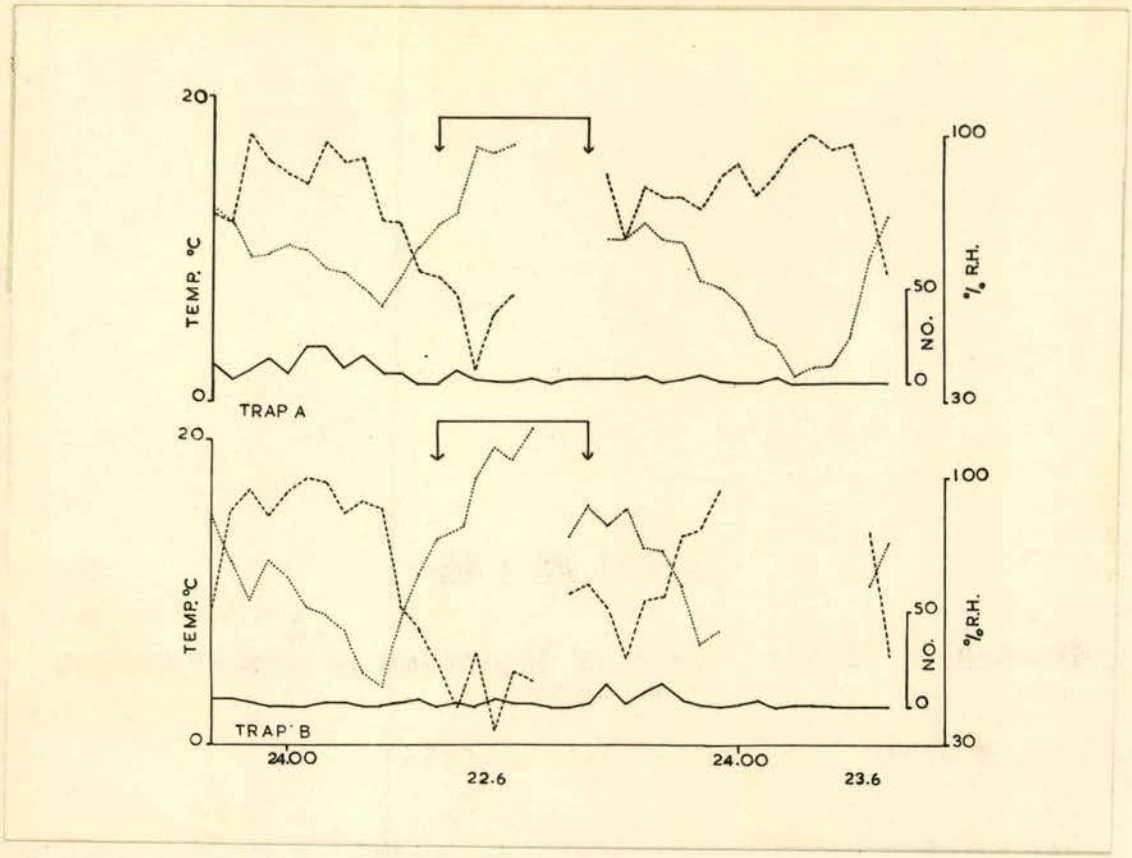



Figure 24

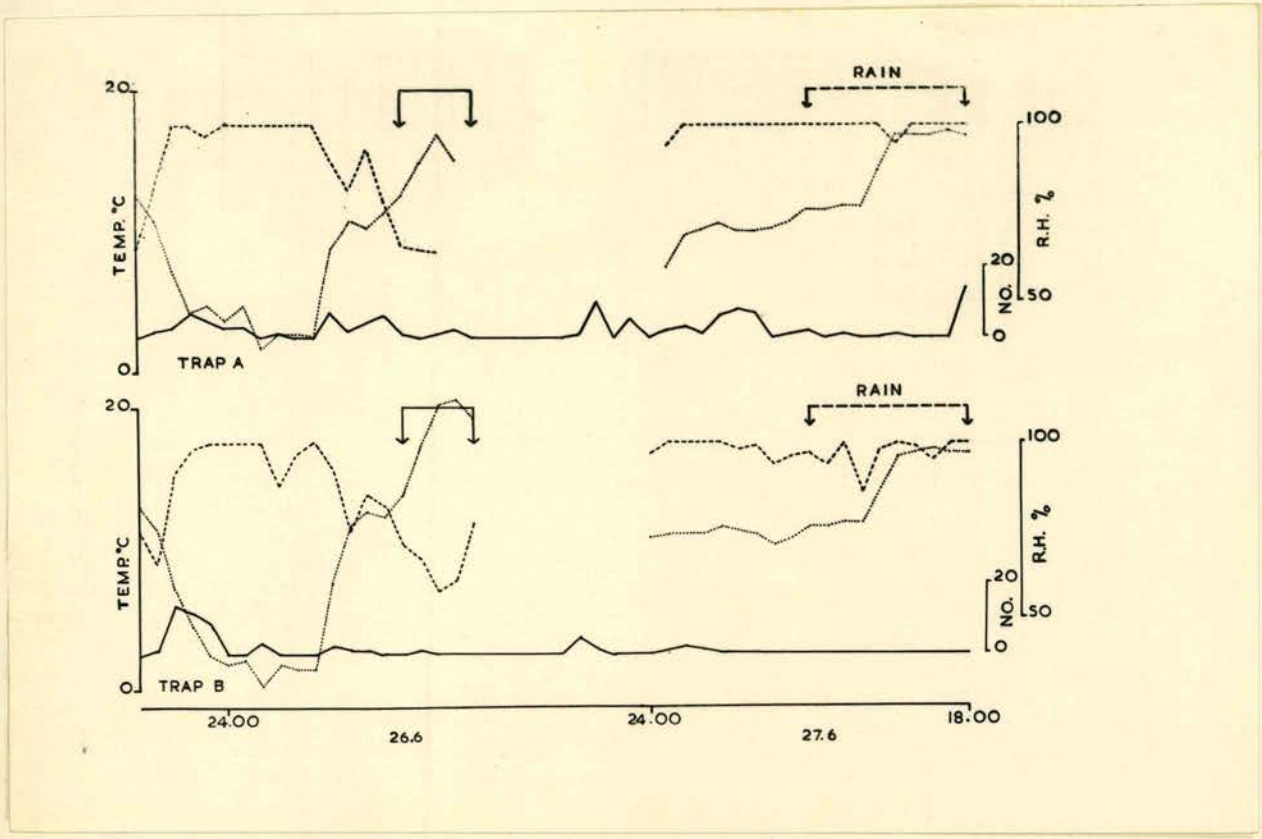
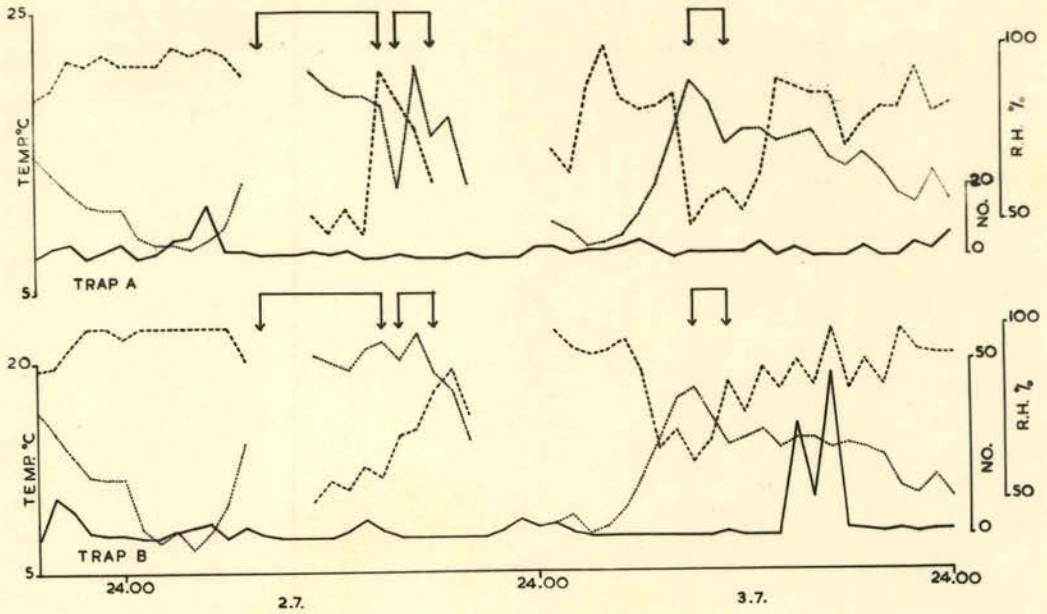



Figure 25

Figures 24 - 29.

| | |
|---|---|
| Continuous line: | Activity expressed as number caught in an hour. |
| Broken line: | Relative humidity. |
| Dotted line: | Temperature, $^{o}C$. |

Hours when radiation was above threshold are indicated by continuous line and arrows.


25
TEMP°C
5
TRAP A
100
R.H. %
20
NO.
0
50
20
TEMP. °C
5
TRAP B
50
NO.
0
100
R.H. %
50
24.00
2.7.
24.00
3.7.
24.00

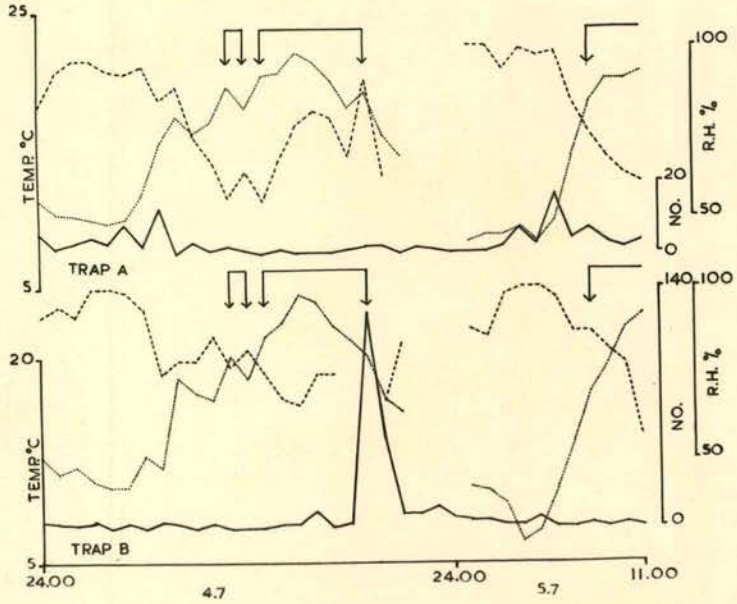

25
TEMP. °C
5
TRAP A
100
R.H. %
20
NO.
0
50
140
100
20
TEMP°C
5
TRAP B
NO.
0
R.H. %
50
24.00
4.7
24.00
5.7
11.00

Figure 26

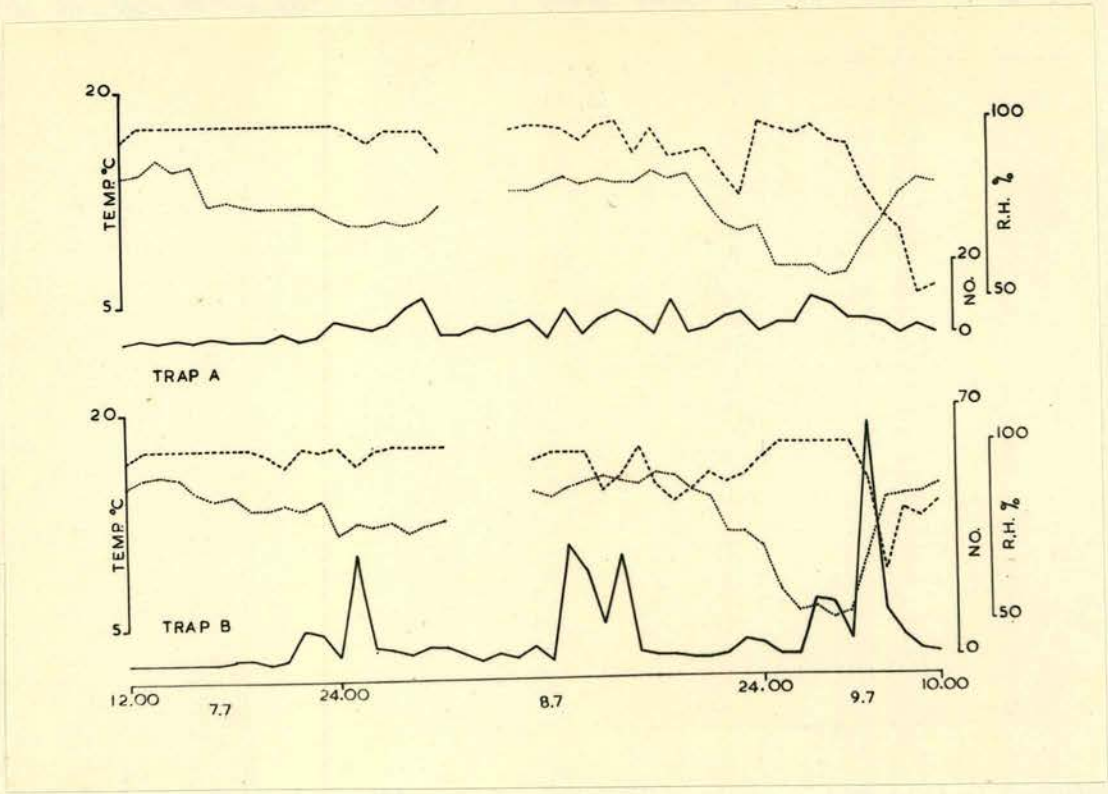



Figure 27

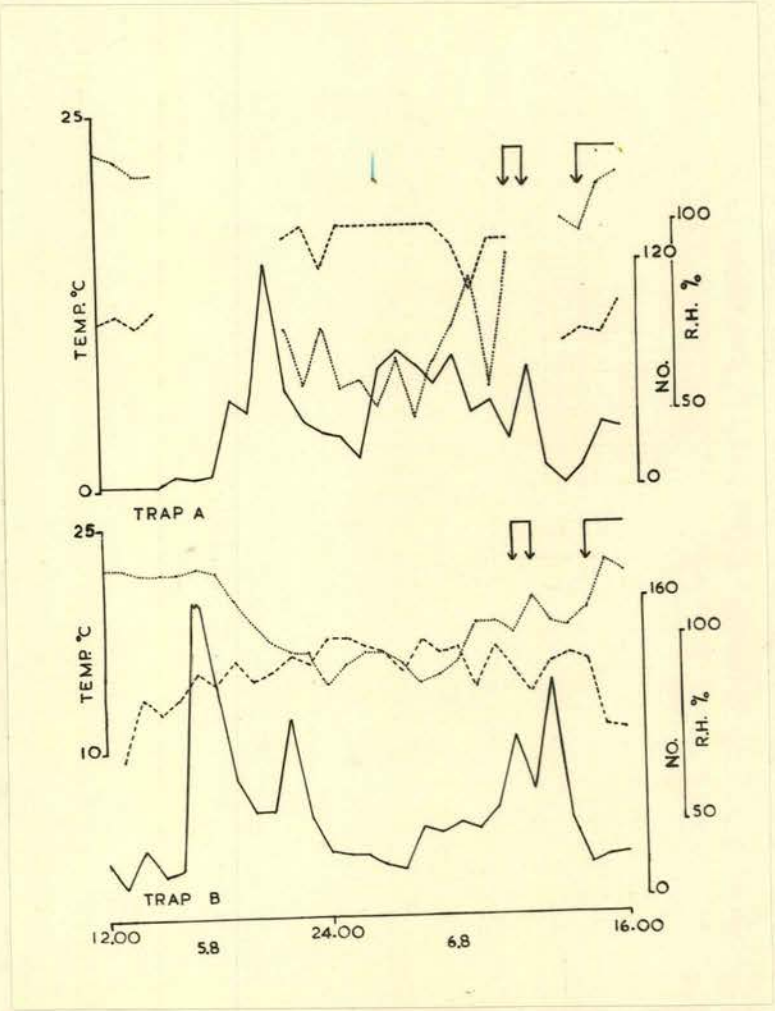



Figure 28

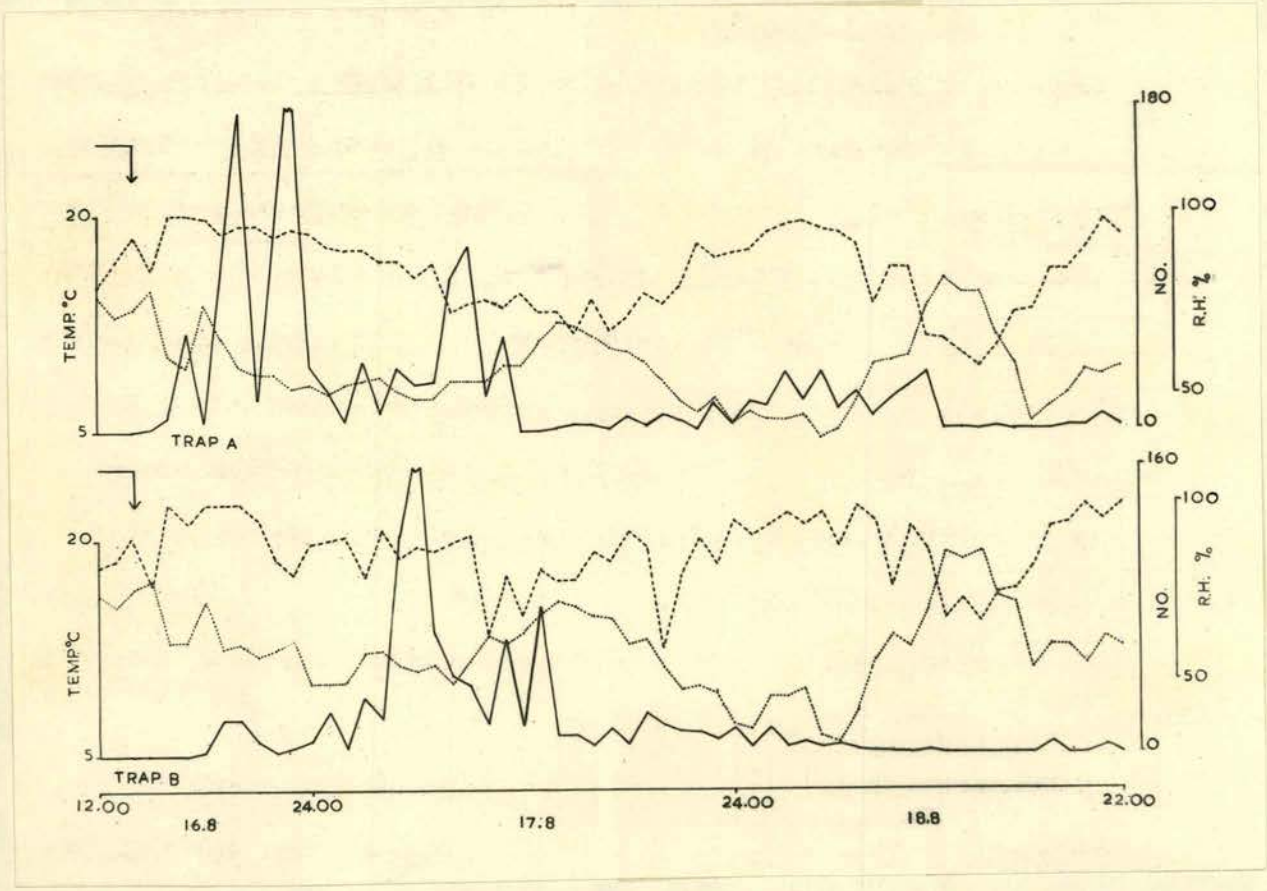

TEMP.°C
20
5
TRAP A
180
100
50
0
NO.
R.H. %
TEMP°C
20
5
TRAP B
160
100
50
0
NO.
R.H. %
12.00
16.8
24.00
17.8
24.00
18.8
22.00

Figure 29

and the hourly means were compared with activity. Six experimental periods, each extending over a few days, were selected. Wind has been shown to have an effect on the activity of C. impunctatus. Since it did not prove possible to measure wind-speed accurately at different levels, windless periods as far as possible were selected for the present studies. Radiation is also known to affect activity. In the previous section it was shown that there is a radiation threshold above which activity is inhibited, but below which radiation does not appear to be affecting the catch. Accordingly, all hours for which the radiation was above threshold (23.4 cal./cm.$^2$/hr., see previous section) were omitted from consideration. Figures 24 to 29 show the data on which computations for the regressions with temperature and humidity were based.

Temperature and humidity are themselves negatively correlated. The method of multiple regression was therefore applied to the data. Each hour, with its readings for temperature in degrees Centigrade, relative humidity, and activity, was taken as a unit for purposes of correlation. Some hours had to be omitted because one or more channels on the recorder used were not functioning. Levels A and B were treated separately since it was not known what climatic factors affect the height at which midges fly, or whether there

was likely to be any interaction between levels.

The six experimental periods were taken at different times during the season. In order to eliminate seasonal effect multiple regressions were applied to the data for each experiment separately. Since each period extended over a few days only, it seems unlikely that the population level would change appreciably within an experiment. The different catches for different hours, therefore, were an expression of activity change. Logarithms of actual numbers were used, to avoid difficulties due to asymmetry. The results obtained were as follows:

Level A. Temperature

| Experiment number | Number of observations (n). | Partial regression coefficient (b) | ($t^2$) | Sampling variance (V) | Weighted b (W) |
|---|---|---|---|---|---|
| 1 | 27 | 0.817 | 7.733** | 0.862 | 11.600 |
| 2 | 33 | - 0.102 | 0.447 | 0.023 | 46.863 |
| 3 | 54 | - 0.058 | 0.060 | 0.054 | 18.376 |
| 4 | 40 | - 0.634 | 7.560** | 0.532 | 18.808 |
| 5 | 14 | - 0.394 | 0.904 | 0.172 | 5.819 |
| 6 | 58 | - 0.300 | 0.748 | 0.120 | 8.334 |

* denotes a probability of 5 %
** " " " " 1 %
*** " " " " 0.1 %

It is evident that the individual regressions for experiments are very variable. The weighted individual regressions were therefore used in a combined test of variance which tested simul-

taneously whether regressions were consistent and whether the correlation between activity and temperature differed significantly from zero. The weight of an individual regression was taken to be the reciprocal of the Sampling Variance (V). The weighted mean was $B = \Sigma Wb/\Sigma W$. The significance of B was tested by $X^2 = B\Sigma Wb$ with one degree of freedom. The heterogenicity $X^2$ was $\Sigma Wb^2 - B\Sigma Wb$ with (n - 1) degrees of freedom.

The $X^2$ analysis for regression of activity on temperature at level A was as follows:

B = -0.1198

| | D. f. | $X^2$ | Mean square |
|---|---|---|---|
| B | 1 | 1.5195 | 1.5195 |
| Heterogenicity | 5 | 15.9349 | 3.1870 |

Variance Ratio (F) = 0.476

Insignificant.

The null hypothesis is therefore not to be rejected and temperature and activity are therefore not significantly related at level A.

Level A. Humidity.

| Experiment number. | (n) | (b) | ($t^2$) | (V) | (W) |
|---|---|---|---|---|---|
| 1 | 27 | 0.156 | 2.228 | 0.011 | 91.827 |
| 2 | 33 | - 0.027 | 0.107 | 0.007 | 142.146 |
| 3 | 54 | 0.041 | 0.52 | 0.003 | 304.878 |
| 4 | 40 | - 0.021 | 0.04 | 0.011 | 88.496 |
| 5 | 14 | 0.232 | 1.329 | 0.040 | 24.722 |
| 6 | 58 | 0.197 | 6.083* | 0.006 | 157.480 |

B = 0.07145

$X^2$ analysis

| | D. f. | $X^2$ | Mean square |
|---|---|---|---|
| B | 1 | 4.1330 | 4.1330 |
| Heterogenicity | 5 | 6.1748 | 1.2350 |

F = 3.347*-
On verge of significance.

Level B Temperature

| Experiment number | (n) | (b) | ($t^2$) | (V) | (W) |
|---|---|---|---|---|---|
| 1 | 20 | 0.296 | 1. | 0.071 | 14.075 |
| 2 | 34 | - 0.254 | 5.669* | 0.011 | 88.137 |
| 3 | 55 | 0.130 | 0.288 | 0.059 | 16.972 |
| 4 | 40 | - 0.572 | 4.337* | 0.076 | 13.238 |
| 5 | 21 | 0.863 | 3.108 | 0.240 | 4.171 |
| 6 | 58 | - 0.980 | 10.714** | 0.090 | 11.161 |

B = - 0.2090

$X^2$ analysis

| | D. f. | $X^2$ | Mean square |
|---|---|---|---|
| B | 1 | 6.4546 | 6.4546 |
| Heterogenicity | 5 | 18.8972 | 3.7790 |

F = 1.708
Insignificant.

Humidity

| Experiment number | (n) | (b) | ($t^2$) | (V) | (W) |
|---|---|---|---|---|---|
| 1 | 20 | - 0.131 | 5.142* | 0.003 | 300.300 |
| 2 | 34 | - 0.025 | 0.143 | 0.004 | 230.415 |
| 3 | 55 | - 0.049 | 0.306 | 0.008 | 128.041 |
| 4 | 40 | - 0.215 | 3.193 | 0.014 | 69.013 |
| 5 | 21 | 0.177 | 1.046 | 0.030 | 33.434 |
| 6 | 58 | - 0.191 | 4.693* | 0.008 | 129.032 |

$B = -0.0953$

$X^2$ analysis

| | D. f. | $X^2$ | Mean square |
|---|---|---|---|
| B | 1 | 8.0857 | 8.0857 |
| Heterogenicity | 5 | 6.4407 | 1.2881 |

$F = 6.278^*$

Significant.

There is seen to be therefore, no significant regression of activity on temperature either at 6 ft. or at 3 ft. Humidity on the other hand seems to have an effect on activity. A positive correlation which is on the verge of significance was obtained at the 6 ft. level while at the 3 ft. level there is a significant negative correlation. If a single year's data is to be regarded as typical, there would therefore seem to be grounds for believing that midges fly higher above the ground when the humidity is high, and descend nearer the ground when conditions are drier. This might explain why in some hours when there are fairly high catches in one trap, the other trap has a low catch. This occurred quite commonly, and can for example be seen in figure 29 where from 16.00 on the 16th August to midnight the catch was predominantly in trap A, while between 4.00 and 7.00 on the 17th, catches were very high in trap B and relatively low in A.

For both levels, catches of more than 10 midges in an hour were only obtained when the relative humidity was above 60 %, and catches of over 100 per hour between 75 % and 97 %. There was one period of rain, on June 27th, when between 9.00 and 18.00 there was a series of heavy showers. Over this period very few midges were captured, as can be seen from figure 25. The explanation of why no catches of over 100 were obtained at 100 % R.H. may be that midges avoid actual precipitation.

Summary.

1. Trapping was performed at three levels, 6 ft., 3 ft., and 6 in. above the ground. There was no significant difference over the season between numbers of C. impunctatus caught at 6 ft. and 3 ft. but significantly a smaller catch was obtained at the 6 in. level.

2. Although the hours of the day differed significantly as regards midge activity, no well defined daily cycle of activity emerged from the analysis.

3. A bimodal seasonal distribution was obtained for the year 1957. A smooth curve was fitted to the data, and five-day running means were also taken, as alternative methods of obtaining seasonal trends by eliminating the

effects of weather conditions.

4. Wind was found to affect the numbers of midges taken in a day. A negative correlation coefficient was obtained between run of wind in 24 hours and daily activity.

5. A radiation threshold was found to exist, above which there was little activity, but below which radiation did not appear to be affecting activity.

6. Temperature did not affect activity. A significant positive regression of activity on humidity was demonstrated for the 6 ft. level, and a significant negative regression for the 3 ft. level. It seemed likely therefore that midges flew higher when the humidity was high.

-o-o-

## GENERAL DISCUSSION

The problem of obtaining an unbiassed estimate of insect activity in the field has been approached by various methods by various workers. Several methods of trapping have been used, some of the main ones being: traps using a food bait (e.g. Dyson-Hudson, 1956, working on Drosophila subobscura and D. obscura); sweep-netting (e.g. Hughes, 1955, working with Meromyza variegata); sticky traps (Broadbent et al., 1948, for work on species of aphids); light traps (e.g. Williams, 1937, 1939 and 1940). With blood-sucking insects, where the presence of the experimenter is equivalent to providing bait, the choice of trapping method is especially important. Davies (1952) in his study of the activity of Simulium spp. subdivided "activity" into flying, attraction, landing and biting. He found that different aspects of behaviour were affected differently by meteorological conditions. Biting and flying increased and landing decreased with rapidly changing pressures. Flight was optimum from 60° to 80° F. at low but not zero saturation deficiencies. Landing was optimum from 65° to 70° F. at zero to intermediate saturation deficiencies. Halcrow (1956) found that the biting cycle of Anopheles gambiae was 1 to 2 hours later in its peak than

the general activity cycle. These findings suggest that a confused picture is likely to emerge when results obtained by different sampling methods are compared, unless the different behavioural responses being sampled are clearly kept in mind.

Most of the main trapping methods have been applied to the study of the seasonal and daily cycle of Culicoides impunctatus. Hill (1947) used attraction to a coloured cloth, thus utilising an optical response. Kettle also relied on this principle by using a black background to his sticky traps, the attractiveness of black to Culicoides having been demonstrated by Hill (Kettle, 1951 a). Parker's (1949) studies were made on the basis of sweepnet data. With a sweepnet he was estimating numbers flying, and the factor of attraction to a host was introduced by his own presence. Kettle (1957) trapped on human bait. Nicholas (1953) did the same in his studies on C. austeni and C. grahamii. In the present studies the suction-trap devised by Johnson (1950) and used by him in estimating the serial population of Aphis fabae was used throughout. The use of this trap eliminated the human factor, both as bait and as part of the trapping, thus reducing experimental error. Insects were sucked in passively so that numbers

caught represented numbers flying in a constant volume of air over a standard period of time. Flying behaviour was therefore being sampled.

Dissections showed that the catch was composed almost entirely of females with ovaries at stages 1 and 2 and without blood in the gut. Stages 3 to 5, which were found to occur only after a blood meal had been taken, were caught very rarely. It was inferred that after a meal had been taken females found resting places and did not fly actively again until they had matured their eggs, oviposited, and were again ready to take a blood meal. It was also shown that C. impunctatus must mate at an early stage, before flying. In investigating flight behaviour then, we are dealing with the behaviour of females which have already mated, have ovaries at stages 1 and 2, and are in search of a blood meal. That mating behaviour was not involved was shown both by the fact that hardly any males were taken in the traps and that almost all females caught flying, and dissected, had already been fertilised.

The population sampled was made up of newly emerged females flying for the first time, females which had oviposited and had newly returned for another blood meal, and females which had failed to obtain a blood meal on their first flight and had returned to fly again.

The actual catch of insects has been related to population by Williams (1940), Catch $\propto$ (activity due to temperature x activity due to humidity x activity due to wind etc.) x population. The size of the catch is therefore partly dependent on population level. The population was found to fluctuate in a bimodal manner over the season in the year studied, and a correction for seasonal trends had therefore to be made in all studies on activity

The importance of continuous recording of microclimatic conditions when comparisons with activity are to be made has long been recognised. In the space of an hour meteorological conditions can fluctuate considerably and readings taken once during an hour may bear no relation to the rest of the hour and consequently to the activity over that hour. Radiation and wind recordings were continuous but with temperature and humidity the hourly means of readings taken at fifteen minute intervals were used. Trapping was carried out over open grassland and the vertical profiles of temperature and humidity obtained were very similar to those described by Geiger (1950) at different times of day.

Wind has the most obvious effect on the activity of the midge, there being a negative correlation between the size of the daily catch and the run of wind. This

agrees with the findings of Williams (1940), who analysed the numbers of all insect species caught at a light trap over four years and found a significant negative correlation between numbers taken in a night and wind. Pratt et al.(1949) found that the activity of Culicoides tristriatulus was inhibited by wind-speeds above 3.5 miles per hour, but that counts were higher at wind-speeds between 1.5 and 1.9 m.p.h. than at wind-speeds lower than this. Laboratory studies by Digby (1958) on Calliphora erythrocephala showed that winds up to 0.7 metres per second had an activating effect but above this value it inhibited flight. The effect of very low wind-speeds on Culicoides impunctatus would not be detected by the methods used in the present study since the run of wind was being taken instead of the wind velocity, so that though the overall inhibitory effect of wind has been demonstrated, the possibility that low winds may stimulate activity has not been investigated.

The fact that C. impunctatus was crepuscular in its activity (Parker, 1949) led to the idea that activity might be optimum at low light intensities. Kettle in 1957 only took this species when light intensity was low. In the present work it has however been shown that activity during hours of total darkness is not significantly

lower than activity during hours of low light intensity. It seems more reasonable to believe that a light threshold exists above which activity is lowered, but below which other climatic factors become more important. A relationship of this type was found by Platt et al. (1957) in laboratory studies on Anopheles quadrimaculatus. There was a light threshold at 500 foot candles with no consistent population response to intensity charges above or below. On the other hand Digby (1958) stated that flight activity in Calliphora erythrocephala was low in the dark, but was independent of light up to 10 lux. after which there was an increase with light until the heating effects of radiation became limiting. Similarly Kettle found that the activity of Chrysops silacea increased with increasing light intensities. In some photopositive insects at least, therefore, there is a regression of activity on light.

The effect of temperature on insect activity has been extensively studied. Laboratory studies have been made on a number of nocturnal species. Thomson showed in 1938 that the behaviour of females of Culex pipiens fatigans differed at different stages of the gonotrophic cycle. Newly emerged females showed avoidance of high

temperatures above 25° C. and a weak avoidance of low temperatures, while hungry females avoided high temperatures but not low. Platt et al. (1957) found that females of Anopheles quadrimaculatus showed maximum activity between 37° C. and 40° C. It is more difficult to determine the effect of temperature in the field, since the cycle of humidity changes is closely related to the temperature cycle. For all species of insect caught at a light trap Williams (1940) showed that the effect of a rise of 5° F. in minimum temperature was to double the night's catch, while a rise of 7° F. in maximum temperature was required to double the catch. In the present study a significant regression of activity on temperature could not be demonstrated. This could mean that temperature was not affecting the activity of C. impunctatus to any significant extent, or that temperature had a different effect on activity at different times during the season. A relationship of this kind was described by Dyson-Hudson (1956) for Drosophila subobscura whose behaviour in summer is closely correlated with light intensity while temperature has only a subsidiary effect, but in early spring and late autumn the position is reversed. Another possibility, which was not tested

for, was that change of temperature affected activity rather than absolute temperature.

With humidity, different species of insects differ widely in their tolerance. Thomson (1938) found that Culex pipiens fatigans at all stages showed a strong avoidance of humidities above 95 %. There was a slight but regular avoidance of low humidities, provided the mosquitoes were offered a humidity range of at least 40 % R.H. Working with Anopheles quadrimaculatus Platt (1957) found the optimum range for activity lay between 70 and 80 % R.H. with a fall off above and below. Humidity was shown to affect the activity of C. impunctatus, there being a tendency for this species to fly higher under damper conditions. It is interesting in view of the avoidance of high and of low humidities demonstrated for mosquito species to find that catches of above 10 midges per hour were not obtained below 60 % R.H. and catches above 100 per hour only between 75 and 97 % R.H.

As it has been shown that various physical factors affect midge activity, the question arises to what extent they explain the diurnal activity cycle. Parker (1949) described a well defined rhythm of activity, with peak

activity twice a day at times related to the times of sunset and sunrise. This was not entirely borne out by the present work. Though there was a tendency for midges to be most active at times related to sunset and sunrise, they could, under suitable conditions be active during the day or during the night. Harker (1958) reviewed the activity cycles that are known for insects, and concluded that in most cases the endogenous rhythm was modified by climatic factors. She states:

> Although light intensity appears to have such a noticeable effect on rhythms, it seems to be the alternation of light and darkness which is the major factor determining the form of the rhythmicity in all the ..... animals (described) above.

Lumsden (1952) explained the bimodal rhythmicity of some West African species of mosquitoes in terms of the differential activity of different age-groups of mosquitoes. Haddow (1956) however took the view that

> ..... Mosquitoes should be considered as being inhibited from biting by unfavourable microclimate and that with the return of favourable conditions they were merely, as it were, released. Whether or not they then began to bite must depend, it was suggested, on some factor connected, perhaps, with age or physiological state.

Culicoides impunctatus would seem to fit the explanation outlined above. Normally radiation would be limiting during the day and when the threshold was passed at a time related to the time of sunset the midges would be released to fly. Humidity has been shown to affect activity, and activity after sunset may be expected to increase with humidity to a maximum. Humidity may be the factor which determines the timing of the peak, since it is not always at the same time with regard to sunset. (Parker, 1949). Temperature does not seem to affect activity, so some other factor must be sought to explain why activity declines as a rule, but increases again at sunrise. This may be due to the fact that there are two groups in the population, one of which is ready to fly at sunset, while the other only becomes ready by sunrise. Nothing is known about the proportions of different age-groups flying at different times, so it is not possible to speculate further on this possibility. Alternatively, midges may respond to the rate of change of a physical factor as well as to its absolute value. Such a situation is described by Digby (1957) for Calliphora erythrocephala with regard to light. He showed that when kept at a high light intensity and then subjected to a low one, there was a sharp drop in activity

followed by recovery due to adaptation. The state of low activity induced by low light intensity persisted when there was a change to a high light intensity and adaptation was gradual. Sharp changes in activity following changes in conditions followed by adaptation would not only account for the increased activity at dawn and dusk, but for the drop off of activity during the night although conditions remain favourable and for activity during the night and during the day when conditions change in a favourable direction. Wind would have an overall inhibitory effect on activity at any part of the cycle.

The patterns of activity obtained at different levels above the ground were not identical, nor were they consistent. Haddow (1956) described differential activity cycles at different heights for Taeniorhynchus fuscopennatus. These were however consistent, cycles being shorter but more intense in the canopy than in the under-storey, and in the under-storey than at ground level. In C. impunctatus the vertical movement has been shown to be related at least partly to humidity, activity being at the higher level when humidity was higher. The possibility that air

currents and wind were playing a part in determining the height at which midges flew must also be kept in mind. Berzina (1953) found that wind affected the height at which species of Simulium were active, depressing activity at unfavourable temperatures and stimulating it at favourable ones. Thus an interaction of climatic factors is likely to be affecting the vertical distribution.

The work on the effect of climatic factors on Culicoides impunctatus has been based on the data obtained in the year 1957. A single year's data is not sufficient to make generalisations, and much further work is required to confirm the conclusions drawn.

-o-o-

## REFERENCES

BATES, M.
The Natural History of Mosquitoes.
New York: Macmillan. (1949).

BECKER, P.
Observations on Culicoides circumscriptus Kieffer (Diptera, Ceratopogonidae=Heleidae).
Ph.D. Thesis. University of Glasgow. (1956).

BERZINA, A.N.
Attack by Simuliids on Man in Nature.
Parasit. Sborn., 15, 353-385. (1953)
In Russian. English Summary in Rev. appl. Ent., B, 42. 180-187. (1956).

BEST, A.C., KNIGHTING, E., PEDLOW, R.H. & STORMONTH, K.
Temperature and Humidity Gradients in the first Hundred Metres at Rye. A paper of the Meteorological Research Committee, London. M.R.P., No. 546. (1950).

BLACKWELL, M.J.
Five years of continuous recording of Total and Diffuse Solar Radiation at Kew Observatory. A paper of the Meteorological Research Committee, London. M.R.P., No. 895. (1954).

BROADBENT, L., DONCASTER, J.P., HULL, R. & WATSON, M.A.
Equipment used for trapping and identifying alate Aphides.
Proc. R. ent. Soc. Lond., A, 23, 57-58. (1948).

BUCKLEY, J.J.C.
On the Development in Culicoides furens Poey, of Filaria (= Mansonella) ozzardi Manson, 1897
J. Helminth., II, 257-258. (1934).

CAMERON, A.E., CREW, F.A.E., DOWNES, J.A., MORISON, G.D. & PEACOCK, A.D.
Control of Midges, an Interim Report of a Sub-Committee of the Scientific Advisory Committee.
H.M.S.O., Edinburgh. (1946).

The Second Report on Control of Midges.
H.M.S.O., Edinburgh. (1948).

CHRISTOPHERS, S.R.
The development of the egg follicle in Anophelines. Paludism, 2, 73-88. (1911).

CLEMENTS, A.N.
Hormonal Control of Ovary Development in Mosquitoes. J. Exp. Biol., 33, 211-223. (1956).

CORBET, P.S.
Seasonal Regulation in British Dragonflies. Nature, 174, 655. (1954).

A Critical response to changing length of day in an insect. Nature, 175, 338. (1955).

Environmental Factors influencing the Induction and Termination of Diapause in the Emperor Dragonfly Anax imperator Leach (Odonata: Aeschnidae). J. Exp. Biol., 33, 1-14. (1956).

DAVIDSON, G.
Measurement of the Ampulla of the Oviduct as a Means of Determining the Natural Daily Mortality of Anopheles gambiae. Ann. trop. Med. Parasit., 49, 24-36. (1955).

DAVIES, D.M.
The Population and Activity of adult Female Blackflies in the Vicinity of a Stream in Algonquin Park, Ontario. Canad. J. Zool., 30, 287-321. (1952).

DAVIES, W.M.
Studies on aphides infesting the Potato crop. VII. Report on a Survey of the aphis population of potatoes in selected districts of Scotland (25 July - 6 August 1936). Ann. appl. Biol., 26, 116-134. (1939).

DETINOVA, T.S.
On the Influence of Glands of Internal Secretion upon the Ripening of the Gonads and the Imaginal Diapause in Anopheles maculipennis. Zool. Zh., 24, 291-298. (1945)
In Russian, with summary in English.

DIGBY, P.S.B.
Flight activity in the blowfly Calliphora erythrocephala in relation to light and radiant heat, with special reference to adaptation.
J. Exp. Biol., 35, 1-19. (1958 a)

Flight activity in the blowfly Calliphora erythrocephala in relation to wind speed, with special reference to adaptation.
J. Exp. Biol., 35, 776-795. (1958 b).

DOWNES, J.A.
Habits and Lifecycle of Culicoides nuxbeculosus Mg.
Nature, 166, 510-511. (1950).

Observations on the Swarming Flight and Mating of Culicoides (Diptera: Ceratopogonidae).
Trans. R. ent. Soc. Lond., 106, 213-236. (1955)

DUKE, B.O.L.
The Uptake of Microfilariae of Acanthocheilonema streptocerca by Culicoides grahamii and their subsequent development.
Ann. trop. Med. Parasit., 48, 416-420. (1954).

DYSON-HUDSON, V.R.D.
The Daily Activity Rhythm of Drosophila subobscura and D. obscura.
Ecology, 37, 562-567. (1956).

EDWARDS, F.W.
Ceratopogonidae.
In EDWARDS, F.W., OLDROYD, H. & SMART, J., British Bloodsucking Flies.
London: British Museum. (1939).

FRAENKEL, G.S. & GUNN, D.L.
The Orientation of Animals.
Oxford: Clarendon. (1940).

GEIGER, R.
The Climate near the Ground.
Massachusetts: Harvard University Press. (1950).

GILLET, J.D.
Initiation and Promotion of Ovarian Development in the mosquito Aedes (Stegomyia) aegypti (Linnaeus). Ann. trop. Med. Parasit., 50, 375-380. (1956).

GOETGHEBUER, M.
Ceratopogonidae de Belgique. Mem. Mus. Hist. Nat. Belg. 8, 1. (1920). Quoted by EDWARDS, (1939).

HADDOW, A.J.
The Mosquitoes of Bwamba County, Uganda. II. Biting Activity with Special Reference to the Influence of Microclimate. Bull. ent. Res., 36, 33-73. (1945).

Rhythmic Biting Activity of Certain East African Mosquitoes. Nature, 177, 531-532. (1956 a).

Observations on the Biting-habits of African Mosquitoes in the Genus Eretmapodites Theobald. Bull. ent. Res., 46, 761-772. (1956 b).

HALCROW, J.C.
Ecology of Anopheles gambiae Giles. Nature, 177, 1103-1105. (1956).

HARKER, J.E.
Diurnal Rhythms in the Animal Kingdom. Biol. Rev., 33, 1-52. (1958).

HARLOW, P.M.
A Study of Ovarial Development and its relation to adult nutrition in the Blowfly Protophormia terraenovae (R.D.) J. Exp. Biol., 33, 777-797. (1956).

HENRARD, C. & PEEL, E.
Culicoides grahamii Austen. Vecteur de Dipetalonema streptocerca et non de Acanthocheilonema perstans. Ann. Soc. belge Méd. trop., 29, 127-143. (1949). Quoted by DUKE. (1954).

HILL, M.A.
The Life-cycle and Habits of Culicoides impunctatus Goet. and C. obsoletus Mg. together with some

observations on the life-cycle of C. odibilis Austen, C. pallidicornis Kief., C. cubitalis Edw. and C. chiopterus Mg.
Ann. trop. Med. Parasit. 41, 55-115. (1947).

The Determination of the hosts of Scottish Culicoides by means of the precipitin technique.
Unpublished report. (1948).

HOPKINS, C.A.
Notes on the Biology of Certain Culicoides studied together with observations on their possible Rôle as Vectors of Acanthocheilonema perstans.
Ann. trop. Med. Parasit., 46, 165-172. (1952).

HUGHES, R.D.
The Influence of the Prevailing Weather on the numbers of Meromyza variegata Meigen (Diptera, Chloropidae) caught with a sweepnet.
J. Anim. Ecol., 24, 324-335. (1955).

JOBLING, B.
The Structure of the Head and Mouth parts in Culicoides pulicaris.
Bull. ent. Res., 18, 211-236. (1928).

On the Bloodsucking midge Culicoides vexans including the description of its eggs and the first-stage larva.
Parasitology, 43, 148-159. (1953).

JOHNSON, C.G.
A Suction trap for small Airborne insects which automatically segregates the catch into successive hourly samples.
Ann. appl. Biol., 37, 80-89. (1950 a)

The comparison of Suction Trap, Sticky Trap and Tow-net for the Quantitative sampling of small airborne insects.
Ann. appl. Biol., 37, 268-285. (1950 b)

The Changing numbers of Aphis fabae Scop., flying at Crop level, in relation to Current weather and to the population on the crop.
Ann. appl. Biol., 39, 525-547. (1952).

JOHNSON, C.G., SOUTHWOOD, T.R.E. & ENTWISTLE, H.
A method for sampling Arthropods and Molluscs from Herbage by Suction.
Nature, 176, 559. (1955).

JOHNSON, C.G. & TAYLOR, L.R.
Periodism and Energy Summation with Special reference to flight rhythms in Aphids.
J. Exp. Biol., 34, 209-221. (1957).

JONES, R.H.
The Laboratory Colonisation of Culicoides variipennis (Coq.)
J. econ. Ent., 50, 107-108. (1957).

KETTLE, D.S.
The Seasonal Distribution of Culicoides impunctatus Goetghebuer with a discussion on the possibility that it may be composed of two or more Biological Races.
Trans. R. ent. Soc. Lond., 101, 125-146. (1950)

The Spatial Distribution of Culicoides impunctatus Goet. under woodland and moorland conditions and its flight range through woodland.
Bull. ent. Res., 42, 239-291. (1951 a).

Some Factors affecting the Population Density and Flight Range of Insects.
Proc. R. ent. Soc. Lond., A, 26, 59-63. (1951 b).

The Effect of Light on the Biting Activity of Chrysops silacea (Diptera, Tabanidae).
Ann. trop. Med. Parasit., 47, 335-339. (1953).

Sex Ratios among British Culicoides.
Proc. R. ent. Soc. Lond., A, 30, 70-72. (1955).

Preliminary observations on weather conditions and the activity of Biting flies.
Proc. R. ent. Soc. Lond., A, 32, 13-20. (1957).

KETTLE, D.S. & LAWSON, J.W.H.
The Early Stages of British Biting Midges, Culicoides Latreille and Allied Genera.
Bull. ent. Res., 43, 421-467. (1952).

LAVEN, H.
Untersuchen und Deutungen zum Culex pipiens complex.
In MATTINGLY et al.
Trans. R. ent. Soc. Lond., 102, 365-368. (1951).

LUMSDEN, W.H.R.
The Crepuscular Biting Activity of Insects in the Forest Canopy in Bwamba, Uganda.
Bull. ent. Res., 42, 721-760. (1952).

MARTINI, E. & TEUBNER, E.
Ueber das Verhalten von Stechmücken, besonders von Anopheles maculipennis, bei verschiedenen Temperaturen und Luftfeuchtigkeiten.
Beih. Arch. Schiffs. - u. Tropenhyg., 37, 1-80 (1933).
Quoted by BATES (1949).

MATTINGLY, P.F., ROZEBOOM, L.E., KNIGHT, K.L., LAVEN, H., DRUMMOND, F.H., CHRISTOPHERS, S.R. & SCHUTE, P.G.
The Culex pipiens Complex.
Trans. R. ent. Soc. Lond., 102, 331-382. (1951)

MEGAHED, M.M.
A Culture method for Culicoides nubeculosus in the Laboratory, with notes on the Biology.
Bull. ent. Res., 47, 107-114. (1956).

MELLANBY, K.
The Daily Rhythm of Activity of the Cockroach, Blatta orientalis. II. Observations and Experiments on a Natural Infestation.
J. Exp. Biol., 17, 278-285. (1940).

MER, G.
The Determination of the age of Anopheles by differences in the Size of the Common Oviduct.
Bull. ent. Res., 23, 563-566. (1932).

NICHOLAS, W.L.
The Bionomics of Culicoides austeni, vector of Acanthocheilonema perstans in the rain-forest of the British Cameroons, together with notes on C. grahamii and other species which may be vectors in the same area.
Ann. trop. Med. Parasit., 47, 187-206. (1953).

PARKER, A.H.
Observations on the Seasonal and Daily Incidence of Certain Biting Midges in Scotland.
Trans. R. ent. Soc. Lond., 100, 179-190. (1949).

Studies on the Eggs of Certain Biting Midges occurring in Scotland.
Proc. R. ent. Soc. Lond., A, 25, 43-52. (1950).

PENMAN, H.L. & LONG, I.
A Portable Thermistor Bridge for micrometeorology among Growing Crops.
J. sci. Instrum., 26, 77-80. (1949).

PFEIFFER, I. W.
The effect of the corpora allata on the metabolism of the adult female grasshopper Melanoplus differentialis.
J. Exp. Zool., 99, 183-233. (1945).

PLATT, R.B., COLLINS, C.L. & WITHERSPOON, J.P.
Reactions of Anopheles quadrimaculatus Say to Moisture, Temperature and Light.
Ecol. Monogr., 27, 303-324. (1957).

PRATT, R.L., MORRIS, B.L. & DALEY, G.J.
Climatic studies: Effect of meteorological factors in Culicoides activity.
Interim Report No. 0-137. Progress Report of the Alaska Insect Project for 1948. 68-70. (1949).

RIDER, N.E. & ROBINSON, G.D.
A Study of the Transfer of Heat and Water Vapour above a surface of short grass.
A paper of the Meteorological Research Committee, London, M.R.P., No. 547. (1950).

RIEK, R.F.
Studies on Allergic Dermatitis (Queensland Itch) of the Horse: the Aetiology of the Disease.
Austr. J. agric. Res., 5, 109-129. (1954).

SHARP, N.A.D.
Filaria perstans; its development in Culicoides austeni.
Trans. R. Soc. trop. Med. Hyg., 12, 371-396. (1928).

SNEDECOR, G.W.
Statistical Methods.
4th ed., Iowa: Iowa State College Press. (1937).

SNOW, W.E.
Feeding Activities of some Blood-sucking Diptera with reference to Vertical Distribution in Bottomland Forest.
Ann. ent. Soc. Amer., 48, 512-521. (1955).

STEWARD, J.S.
Onchocerca cervicalis (Raillet and Henry, 1910) and its Development in Culicoides nubeculosus Mg.
Univ. Camb. Inst. Anim. Path., 3, 272-284. (1933).
Quoted by EDWARDS (1939).

TATE, P. & VINCENT, M.
The Biology of Autogenous and Anautogenous races of Culex pipiens L.
Parasitology, 28, 115-144. (1936).

TAYLOR, L.R.
An improved suction trap for insects.
Ann. appl. Biol., 38, 582-591. (1951).

Temperature Relations of General Development and Behaviour in Aphis fabae Scop.
J. Exp. Biol., 34, 189-208. (1957)

THOMAS, I. & VEVAI, E.J.
Aphis migration. An analysis of the results of five seasons' trapping in North Wales.
Ann. appl. Biol., 27, 393-405. (1940).

THOMSON, R.C.M.
The Reactions of Mosquitoes to Temperature and Humidity.
Bull. ent. Res., 29, 125-140. (1938).

Mosquito Behaviour in Relation to Malaria Transmission and Control in the Tropics.
London: Arnold. (1951).

DU TOIT, R.M.
The Transmission of Blue-Tongue and Horse-Sickness by Culicoides.
Onderstepoort J. vet. Sci., 19, 7-16. (1944).

WIGGLESWORTH, V.B.
The function of the corpus allatum in the growth and reproduction of Rhodnius prolixus (Hemiptera). Quart. J. micr. Sci., 79, 91-121. (1936).

WILLIAMS, C.B.
The use of Logarithms in the Interpretation of Certain Entomological Problems.
Ann. appl. Biol., 24, 404-414. (1937).

An analysis of four years' captures of insects in the Light Trap. Part I. General Survey: sex proportion; phenology and time of flight.
Trans. R. ent. Soc. Lond., 89, 79-132. (1939)

An analysis of four years' captures of insects in a Light Trap. Part II. Effect of weather conditions on insect activity; and the estimation and forecasting of changes in the insect population.
Trans. R. ent. Soc. Lond., 90, 227-306. (1940).

WOOLF, B.
Computation and Interpretation of Multiple Regressions.
J.R. statist. Soc. B., 13, 100-119. (1951)

-o-o-

APPENDIX:

TABLES 1 TO 10.

## TABLE 1

C.impunctatus, lengths of ova in ovaries of individual specimens. Smallest and largest of 5 measurements recorded. A.u. = Arbitrary units. 1 a.u. = 2.4 μ.

| Date 1956 | Stage: 1 | | 2 | | 3a | | 3b | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. |
| 22/6 | | | 18-20 | 43.2-48.0 | | | | | | | | |
| 27/6 | 25 | 60.0 | 26-27 | 62.4-64.8 | | | | | | | | |
| | | | 24 | 56.7 | | | | | | | | |
| 29/6 | 18-21 | 43.2-50.4 | | | | | | | | | | |
| | 20 | 48.0 | | | | | | | | | | |
| | 27-29 | 64.8-69.6 | | | | | | | | | | |
| | 27-30 | 64.8-72.0 | | | | | | | | | | |
| | 24-27 | 57.6-64.8 | | | | | | | | | | |
| 3/7 | 24-26 | 57.6-62.4 | 21-22 | 50.4-52.8 | | | | | | | | |
| 6/7 | | | 20-21 | 48.0-50.4 | | | | | | | | |
| | | | 19-20 | 45.6-48.0 | | | | | | | | |
| | | | 19-20 | 45.6-48.0 | | | | | | | | |
| | | | 17-19 | 40.8-45.6 | | | | | | | | |
| 7/7 | | | | | | | | | 58-62 | 139.2-148.8 | | |
| 10/7 | 27-28 | 64.8-67.2 | | | 32-34 | 76.8-81.6 | | | | | | |
| | 25-28 | 60.0-67.2 | | | | | | | | | | |

17/7 ...continued on next page.

TABLE 1, continued.

| Date | Stage: 1 | | 2 | | 3a | | 3b | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. |
| 17/7 | 15-17 | 36.0-40.8 | 19-23 | 45.6-55.2 | | | | | 70 | 168.0 | 172-192 | 412.8-460.8 |
| | 20-24 | 48.0-57.6 | 22-25 | 52.8-60.0 | | | | | 50 | 120.0 | | |
| | 20-24 | 48.0-57.6 | 20 | 48.0 | | | | | 92 | 220.8 | 156-160 | 374.4-384.0 |
| | 20-25 | 48.0-60.0 | | | | | | | | | | |
| | 20 | 48.0 | | | | | | | | | | |
| | 20-22 | 48.0-52.8 | | | | | | | | | | |
| | 20-21 | 48.0-50.4 | | | | | | | | | | |
| | 19-21 | 45.6-50.4 | | | | | | | | | | |
| | 10-14 | 24.0-33.6 | | | | | | | | | | |
| | 21-24 | 50.4-57.6 | | | | | | | | | | |
| | 16-17 | 38.4-40.8 | | | | | | | | | | |
| 18/7 | 25 | 60.0 | 24-30 | 57.6-72.0 | | | | | | | | |
| | 27-29 | 64.8-69.6 | | | | | | | | | | |
| | 21-25 | 50.4-60.0 | | | | | | | | | | |
| 24/7 | 18-20 | 43.2-48.0 | 22-25 | 52.8-60.0 | | | | | | | | |
| | 20-21 | 48.0-50.4 | | | | | | | | | | |
| | 18-20 | 43.2-48.0 | | | | | | | | | | |
| 27/7 | 23 | 55.2 | | | | | | | | | | |
| | 20-21 | 48.0-50.4 | | | | | | | | | | |
| | 18-24 | 43.2-57.6 | | | | | | | | | | |
| | 23 | 55.2 | | | | | | | | | | |
| | 20-21 | 48.0-50.4 | | | | | | | | | | |
| | 23-24 | 55.2-57.6 | | | | | | | | | | |
| 6/8 | 11-12 | 26.4-28.8 | | | | | | | | | | |
| 7/8 | | | | | | | | | 55 | 132.0 | | |

## TABLE 2

C. heliophilus, lengths of ova in ovaries of individual specimens. Smallest and largest of 5 measurements recorded. A.u. = Arbitrary units. 1 a.u. = 2.4 μ.

| Stage | 1 | | 2 | | 3a | | 3b | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date 1956 | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. | A.u. | μ. |
| 22/6 | | | 20-22 | 48.0-52.8 | 20-22 | 48.0-52.8 | | | | | | |
| | | | 20-22 | 48.0-52.8 | | | | | | | | |
| 23/6 | 16-18 | 38.4-43.2 | | | | | | | | | | |
| 25/6 | 26-30 | 62.4-72.0 | 32-34 | 76.8-81.6 | | | 35-43 | 84.0-103.2 | 48-50 | 115.2-120.0 | | |
| | 22-27 | 52.8-64.8 | 30-32 | 72.0-76.8 | | | 36-37 | 86.4-88.8 | 45-47 | 108.0-112.8 | | |
| | 25-28 | 60.0-67.2 | 26-29 | 62.4-69.7 | | | 32-39 | 76.8-93.6 | 42-47 | 100.8-112.8 | | |
| | 24-26 | 57.6-62.4 | | | | | | | | | | |
| | 23-27 | 55.2-64.8 | | | | | | | | | | |
| 27/6 | 30-31 | 72.0-74.4 | | | | | | | 69-72 | 165.6-172.8 | | |
| | 26-30 | 62.4 72.0 | | | | | | | 70-72 | 168.0-172.8 | | |
| 28/6 | | | 16-22 | 38.4-52.8 | | | | | | | | |
| | | | 16-19 | 38.4-45.6 | | | | | | | | |
| 29/6 | 24 | 57.6 | | | 31-33 | 74.4-79.2 | | | | | | |
| | 14-17 | 33.6-40.8 | | | | | | | | | | |
| 3/7 | | | | | 23-26 | 55.2-62.4 | | | | | | |
| | | | | | 29-30 | 69.4-72.0 | | | | | | |
| 4/7 | 23-24 | 55.2-57.6 | | | | | | | | | | |
| 6/7 | 19-22 | 45.6-52.8 | | | | | | | 35-43 | 84.0-103.2 | | |
| | 20 | 48.0 | | | | | | | | | | |
| | 19-22 | 45.6-52.8 | | | | | | | | | | |
| | 20-22 | 48.0-52.8 | | | | | | | | | | |

TABLE 3

Ampulla measurements on ♀ C. impunctatus. Diameters of both ampullae in arbitrary units. 1 unit = 2.4 μ.

| Number | Date of capture | Ovary Stage | Presence of blood in gut. | Ampulla diameters. | |
|---|---|---|---|---|---|
| 1 | 20 8 56 | 1 | - | 30 | 26 |
| 2 | | 1 | - | 35 | 45 |
| 3 | | 2 | - | 31 | - |
| 4 | | 1 | - | 30 | 30 |
| 5 | | 1 | - | 30 | 30 |
| 6 | | 1 | - | 30 | 30 |
| 7 | | 2 | - | 30 | 38 |
| 8 | | 2 | - | 35 | 40 |
| 9 | | 1 | - | 30 | 30 |
| 10 | | 2 | - | 42 | 42 |
| 11 | | 1 | - | 35 | - |
| 12 | 21 8 56 | 1 | - | 45 | - |
| 13 | | 1 | - | 38 | - |
| 14 | | 1 | - | 45 | 45 |
| 15 | | 2 | - | 30 | 35 |
| 16 | | 1 | - | 40 | - |
| 17* | | 2 | - | 38 | - |
| 18 | | 2 | + | 28 | - |
| 19 | | 1 | - | 41 | - |
| 20 | | 1 | - | 30 | 25 |
| 21 | | 2 | - | 30 | - |
| 22 | | 1 | - | 40 | - |
| 23 | /... continued on next page. | | | | |

* This specimen had two eggs from a previous egg-laying retained in the oviduct of one side.

TABLE 3, continued.

| Number | Date of capture | Ovary Stage | Presence of blood in gut. | Ampulla diameters. | |
|---|---|---|---|---|---|
| 23 | 22 8 56 | 2 | - | 40 | 40 |
| 24 | | 1 | - | 40 | 35 |
| 25 | | 1 | - | 30 | 35 |
| 26 | | 2 | - | 30 | 30 |
| 27 | | 2 | - | 38 | 38 |
| 28 | | 2 | - | 33 | 34 |
| 29 | | 1 | - | 25 | 27 |
| 30 | | 2 | - | 40 | - |
| 31 | | 1 | - | 30 | 30 |
| 32 | 23 8 56 | 2 | - | 30 | - |
| 33 | | 1 | - | 35 | 36 |
| 34 | | 1 | - | 45 | 45 |
| 35 | | 2 | - | 43 | - |
| 36 | | 1 | - | 30 | 30 |
| 37 | 27 8 56 | 1 | - | 27 | 27 |
| 38 | | 1 | - | 30 | - |
| 39 | | 1 | - | 40 | - |
| 40 | | 2 | - | 35 | - |
| 41 | | 2 | - | 25 | 25 |
| 42 | 28 8 56 | 1 | - | 30 | - |
| 43 | | 2 | - | 32 | 30 |
| 44 | | 1 | - | 45 | - |
| 45 | | 1 | - | 30 | - |
| 46 | | 1 | - | 35 | 35 |
| 47 | | 2 | - | 45 | - |
| 48 | | 1 | - | 40 | 38 |
| 49 | | 1 | - | 42 | - |
| 50 | | 1 | - | 35 | - |
| 51 | | 2 | - | 45 | 47 |

NOTE TO TABLE 3

Ampulla Diameters Grouped into Classes.

| Class Number | Limits, A.u. | Items. |
|---|---|---|
| 1 | 24.5 - 26.5 | 1 |
| 2 | 26.5 - 28.5 | 3 |
| 3 | 28.5 - 30.5 | 14 |
| 4 | 30.5 - 32.5 | 2 |
| 5 | 32.5 - 34.5 | 1 |
| 6 | 34.5 - 36.5 | 7 |
| 7 | 36.5 - 38.5 | 4 |
| 8 | 38.5 - 40.5 | 8 |
| 9 | 40.5 - 42.5 | 3 |
| 10 | 42.5 - 44.5 | 1 |
| 11 | 44.5 - 46.5 | 6 |
| 12 | 46.5 - 48.5 | 1 |

Mean = 35.8 A.u.
Standard deviation = 5.997 A.u.

Assuming that these measurements were normally distributed, probits were used in calculating the expected number of items to each class.

| Class Number | Expected Number (m) | Number Obtained | Deviation (d) | $d^2/m$ |
|---|---|---|---|---|
| 1, 2 & 3 | 8.08 | 18 | + 9.92 | 12.18 |
| 4 | 5.23 | 2 | - 3.23 | 1.99 |
| 5 | 6.28 | 1 | - 5.28 | 4.44 |
| 6 | 6.74 | 7 | + 0.26 | 0.01 |
| 7 | 6.49 | 4 | - 2.49 | 0.96 |
| 8 | 5.44 | 8 | + 2.56 | 1.20 |
| 9 | 4.31 | 3 | - 1.31 | 0.40 |
| 10, 11 & 12 | 5.80 | 8 | + 2.20 | 0.83 |

Chi-square ($x^2$) = 22.01

For 7 D.f., P 0.01

The distribution of measurements differs significantly from the normal.

Note to Table 3, continued.

Assuming that the measurements in Classes 1 to 5 were normally distributed, probits were used in calculating the expected number of items in each class.

Mean = 29.7 A.u. Standard deviation = 3.81 A.u.

| Class Number | Expected Number (m) | Number Obtained | Deviation (d) | $d^2/m$ |
|---|---|---|---|---|
| 1 & 2 | 5.3 | 4 | - 1.3 | 0.32 |
| 3 | 8.7 | 14 | + 5.3 | 3.22 |
| 4 & 5 | 6.8 | 3 | - 3.8 | 2.12 |

$X^2 = 5.66$

For 2 D.f., P 0.10

The distribution of measurements does not differ significantly from a normal distribution.

Assuming that the measurements in Classes 5 to 12 were normally distributed, the expected number in each class was again calculated using probits.

Mean = 39.9 A.u. Standard deviation = 3.81 A.u.

| Class Number | Expected Number (m) | Number Observed | Deviation (d) | $d^2/m$ |
|---|---|---|---|---|
| 5 & 6 | 4.96 | 8 | + 3.04 | 1.86 |
| 7 | 5.29 | 4 | - 1.29 | 0.31 |
| 8 | 6.38 | 8 | + 1.62 | 0.41 |
| 9 | 5.89 | 3 | - 2.89 | 1.42 |
| 10, 11 & 12 | 7.30 | 8 | + 0.70 | 0.07 |

$X^2 = 4.07$

For 4 D.f., P 0.30

The population therefore does not differ significantly from a normally distributed one.

---

TABLE 4

Specimens of C. impunctatus examined in saline for the presence or absence of spermatozoa in the spermatheca.

| No. | Capture: Date 1956 | Capture: Time | Capture: Place | Presence of blood in gut. | Ovary Stage | Presence of Sperm. |
|---|---|---|---|---|---|---|
| 1 | 26/6 | 21.15 | Henhouse | - | 1 | + |
| 2 | | " | " | - | 1 | + |
| 3 | | " | " | - | 2 | + |
| 4 | 6/7 | 21.00 | " | - | 1 | + |
| 5 | | " | " | - | 2 | - |
| 6 | | " | " | - | 1 | + |
| 7 | | " | " | - | 1 | + |
| 8 | 10/7 | 21.00 | " | - | 2 | + |
| 9 | | " | " | - | 2 | + |
| 10 | 18/7 | 10.30 | " | + | 1 | + |
| 11 | | " | " | + | 1 | + |
| 12 | | " | " | + | 1 | - |
| 13 | | " | " | + | 2 | + |
| 14 | 24/7 | 10.30 | " | - | 2 | + |
| 15 | | " | " | - | 1 | + |
| 16 | | " | " | - | 2 | + |
| 17 | | " | " | - | 1 | + |
| 18 | | " | " | + | 1 | + |
| 19 | 26/7 | 20.30-21.00 | Wood, flying | - | 1 | + |
| 20 | | " | " | - | 2 | + |
| 21 | | " | " | - | 2 | + |
| 22 | | " | " | - | 2 | + |
| 23 | | " | " | - | 1 | + |
| 24 | | " | " | - | 2 | + |
| 25 | | " | " | - | 1 | + |
| 26 | 27/7 | 16.00 | " | - | 1 | + |
| 27 | | " | " | - | 2 | + |
| 28 | | " | " | - | 1 | + |
| 29 | | " | " | - | 1 | + |
| 30 | | " | " | - | 2 | + |

TABLE 5

Specimens of C. heliophilus examined in saline for presence or absence of spermatozoa in the spermatheca.

| No. | Capture | | | Presence of blood in gut. | Ovary Stage | Presence of Sperm. |
|---|---|---|---|---|---|---|
| | Date 1956 | Time | Place | | | |
| 1 | 22/6 | 20.00 | Henhouse | - | 2 | - |
| 2 | | " | " | - | 1 | - |
| 3 | | 21.30 | " | + | 3a | - |
| 4 | | " | " | - | 2 | + |
| 5 | | " | " | - | 1 | - |
| 6 | | " | " | - | 1 | - |
| 7 | 23/6 | 14.30 | Wood, flying | - | 1 | - |
| 8 | | " | " | - | 2 | - |
| 9 | 28/6 | 16.15 | " | - | 1 | - |
| 10 | | " | " | - | 2 | - |
| 11 | 6/7 | 20.00 | Roadside swarm | - | 2 | - |
| 12 | | " | " | - | 1 | - |
| 13 | | " | " | - | 1 | ? |
| 14 | | " | " | - | 2 | - |
| 15 | 18/7 | 10.30 | Henhouse | + | 1 | - |

TABLE 6

Distribution of the catch of C. impunctatus between the three traps, A, B and C, over the season in 1957.

| Date | Numbers caught. | | | | Percentage of total | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | Total | A | B | C |
| 20 6 - 21 6 | 155 | 87 | 27 | 269 | 58 | 32 | 10 |
| 21 22 | 163 | 37 | 14 | 214 | 76 | 17 | 7 |
| 22 23 | 21 | 52 | 5 | 78 | 27 | 67 | 6 |
| 25 - 26 | 45 | 44 | 4 | 93 | 48 | 47 | 4 |
| 26 27 | 50 | 10 | 0 | 60 | 83 | 17 | 0 |
| 27 28 6. | 60 | 6 | 0 | 66 | 91 | 9 | 0 |
| 1 7 - 2 7 | 54 | 58 | 14 | 126 | 43 | 46 | 11 |
| 2 3 | 29 | 25 | 10 | 64 | 45 | 39 | 16 |
| 3 4 | 117 | 126 | 27 | 270 | 43 | 47 | 10 |
| 4 5 | 118 | 246 | 28 | 392 | 30 | 63 | 7 |
| 5 6 | 242 | 319 | 77 | 638 | 38 | 50 | 12 |
| 6 7 | 56 | 23 | 2 | 81 | 69 | 29 | 2 |
| 7 8 | 57 | 78 | 8 | 143 | 40 | 55 | 6 |
| 8 9 | 96 | 235 | 35 | 366 | 26 | 64 | 10 |
| 9 10 | 151 | 165 | 56 | 372 | 41 | 44 | 15 |
| 11 - 12 | 9 | 36 | 27 | 72 | 12 | 50 | 38 |
| 12 13 | 22 | 11 | 8 | 41 | 54 | 27 | 19 |
| 13 14 | 1 | 21 | 7 | 29 | 3 | 72 | 24 |
| 18 - 19 | 16 | 62 | 15 | 93 | 17 | 67 | 16 |
| 19 20 | 11 | 13 | 6 | 30 | 37 | 43 | 20 |
| 20 21 | 37 | 25 | 4 | 66 | 56 | 38 | 6 |
| 21 22 | 10 | 4 | 0 | 14 | 71 | 29 | 0 |
| 22 23 | 24 | 12 | 0 | 36 | 67 | 33 | 0 |
| 23 24 | 33 | 4 | 0 | 37 | 89 | 11 | 0 |
| 24 25 | 14 | 8 | 0 | 22 | 64 | 36 | 0 |
| 25 26 | 0 | 0 | 0 | 0 | | | |
| 26 27 | 8 | 5 | 0 | 13 | 62 | 38 | 0 |
| 27 28 | 48 | 24 | 0 | 72 | 67 | 33 | 0 |
| 28 29 | 32 | 5 | 0 | 37 | 86 | 14 | 0 |
| 29 30 | 33 | 10 | - | 43 | | | |
| 30 31 | 50 | 9 | - | 59 | | | |
| 31 7. 1 8 | 30 | 23 | 2 | 55 | 54 | 42 | 4 |
| 1 8 - 2 8 | 44 | 6 | 0 | 50 | 88 | 12 | 0 |
| 2 3 | 53 | 18 | 4 | 75 | 71 | 24 | 5 |
| 3 4 | 494 | 154 | 41 | 689 | 72 | 22 | 6 |
| 4 5 | 588 | 516 | 48 | 1152 | 51 | 45 | 4 |
| 5 / | | | | | | | |

TABLE 6, contd.

| | Date | | Numbers caught. | | | | Percentage of total | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | Total | A | B | C |
| 5 | 8 - 6 | 8 | 1018 | 1321 | 502 | 2840 | 36 | 46 | 18 |
| 6 | 7 | | 1300 | 695 | 36 | 2031 | 64 | 34 | 2 |
| 7 | 8 | | 833 | 334 | 40 | 1207 | 69 | 28 | 3 |
| 8 | 9 | | 4 | 21 | 5 | 30 | 13 | 70 | 17 |
| 9 | 10 | | 3267 | 3184 | 122 | 6573 | 50 | 48 | 2 |
| 10 | 11 | | 694 | 676 | 56 | 1426 | 49 | 47 | 4 |
| 11 | 12 | | 384 | 266 | 9 | 659 | 59 | 40 | 1 |
| 12 | 13 | | 326 | 134 | 22 | 482 | 68 | 28 | 4 |
| 13 | 14 | | 25 | 29 | 0 | 54 | 46 | 54 | 0 |
| 14 | 15 | | 82 | 259 | - | 341 | | | |
| 15 | 16 | | 129 | 66 | - | 195 | | | |
| 16 | 17 | | 1167 | 656 | 58 | 1881 | 62 | 35 | 3 |
| 17 | 18 | | 280 | 267 | 16 | 563 | 50 | 47 | 3 |
| 18 | 19 | | 21 | 17 | - | 38 | | | |
| 19 | 20 | | 32 | 29 | - | 61 | | | |
| 20 | 21 | 8. | 2 | 10 | - | 12 | | | |
| 2 | 9 - 3 | 9 | 37 | 16 | 0 | 53 | 70 | 30 | 0 |
| 3 | 4 | | 15 | 9 | 2 | 26 | 58 | 35 | 8 |
| 4 | 5 | | 564 | 450 | 122 | 1136 | 49 | 40 | 11 |
| 5 | 6 | | 15 | 20 | 1 | 36 | 42 | 56 | 3 |
| 6 | 7 | | 3 | 2 | 0 | 5 | | | |
| 7 | 8 | | 14 | 1 | 0 | 15 | 93 | 7 | 0 |
| 8 | 9 | | 0 | 10 | 0 | 10 | 0 | 100 | 0 |
| 9 | 10 | | 25 | 41 | 0 | 66 | 38 | 62 | 0 |
| 10 | 11 | | 206 | 494 | 0 | 700 | 29 | 71 | 0 |
| 11 | 12 | | 36 | 82 | 0 | 118 | 31 | 69 | 0 |
| 12 | 13 | | 2 | 10 | 0 | 12 | 17 | 83 | 0 |
| 13 | 14 | | 1 | 0 | 0 | 1 | | | |
| 14 | 15 | | 0 | 0 | 0 | 0 | | | |
| 15 | 16 | | 153 | 408 | 0 | 561 | 27 | 73 | 0 |
| 16 | 17 | | 131 | 119 | - | 250 | | | |
| 17 | 18 | | 72 | 52 | - | 124 | | | |
| 18 | 19 | | 27 | 24 | - | 51 | | | |
| 19 | 20 | | 0 | 0 | - | 0 | | | |
| 20 | 21 | | 29 | 80 | - | 109 | | | |
| 21 | 22 | 9. | 2 | 1 | - | 3 | | | |
| | Means: | | 195.2 | 172.7 | 25.2 | | 50.9 | 43.0 | 6.1 |

TABLE 7.

Total numbers of C. impunctatus caught at different times of day between June 20 and September 21, 1957.

| Period | Hours | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12-13 | 13-14 | 14-15 | 15-16 | 16-17 | 17-18 | 18-19 | 19-20 | 20-21 | 21-22 | 22-23 | 23-24 |
| 20/6 - 14/7 | 63 | 103 | 85 | 103 | 90 | 129 | 130 | 277 | 161 | 135 | 147 | 154 |
| 18/7 - 2/8 | 22 | 12 | 7 | 9 | 29 | 48 | 23 | 30 | 41 | 43 | 25 | 22 |
| 2/8 - 20/8 | 483 | 359 | 631 | 791 | 621 | 1353 | 981 | 1425 | 1726 | 1146 | 1662 | 796 |
| 3/9 - 21/9 | 711 | 59 | 94 | 93 | 82 | 90 | 93 | 104 | 119 | 82 | 130 | 274 |
| Hourly Totals: | 1279 | 533 | 817 | 996 | 822 | 1620 | 1227 | 1836 | 2047 | 1406 | 1964 | 1246 |

/continued .....

| ..... Period | ..... Hours | | | | | | | | | | | | 24-Hour Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24-1 | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 | 7-8 | 8-9 | 9-10 | 10-11 | 11-12 | |
| 20/6 - 14/7 | 214 | 181 | 140 | 137 | 247 | 208 | 214 | 129 | 140 | 90 | 83 | 83 | 3,444 |
| 18/7 - 2/8 | 39 | 21 | 37 | 32 | 33 | 33 | 34 | 31 | 25 | 34 | 21 | 51 | 702 |
| 2/8 - 20/8 | 798 | 833 | 602 | 623 | 485 | 667 | 847 | 645 | 490 | 432 | 442 | 659 | 19,497 |
| 3/9 - 21/9 | 177 | 121 | 110 | 118 | 183 | 116 | 83 | 61 | 52 | 66 | 32 | 49 | 3,099 |
| Hourly Totals: | 1228 | 1156 | 889 | 910 | 948 | 1024 | 1178 | 866 | 707 | 622 | 578 | 842 | 26,742 |
| | | | | | | | | | | | | Seasonal Total: | 26,742 |

TABLE 8

Numbers of C. impunctatus caught over the season in 1957, grouped to show seasonal trends.

| Mid-date for Group | Individual Days | Daily Catch (n) | Log. Catch (Log. n + 1) | Mean Log. Catch for Group |
|---|---|---|---|---|
| | 20 6 - 21 6 | 269 | 2.431 | |
| 21 6 - 22 6 | 21 22 | 214 | 2.332 | 2.220 |
| | 22 23 | 78 | 1.898 | |
| | 25 26 | 93 | 1.973 | |
| | 26 27 | 60 | 1.785 | |
| 27 6 | 27 28 | 66 | 1.826 | 1.859 |
| | 28 29 6 | 70 | 1.851 | |
| | 1 7 - 2 7 | 126 | 2.104 | |
| | 2 3 | 64 | 1.813 | |
| 3 7 - 4 7 | 3 4 | 270 | 2.433 | 2.350 |
| | 4 5 | 392 | 2.594 | |
| | 5 6 | 638 | 2.806 | |
| | 6 7 | 81 | 1.914 | |
| | 7 8 | 143 | 2.158 | |
| 8 7 | 8 9 | 366 | 2.565 | 2.302 |
| | 9 10 | 372 | 2.572 | |
| | 11 12 | 72 | 1.862 | |
| 12 7 - 13 7 | 12 13 | 41 | 1.623 | 1.654 |
| | 13 14 | 29 | 1.477 | |
| | 18 19 | 93 | 1.973 | |
| | 19 20 | 30 | 1.491 | |
| 20 7 - 21 7 | 20 21 | 66 | 1.826 | 1.607 |
| | 21 22 | 14 | 1.176 | |
| | 22 23 | 36 | 1.568 | |
| | 23 24 | 37 | 1.580 | |
| | 24 25 | 22 | 1.362 | |
| 25 7 - 26 7 | 25 26 | 0 | 0.000 | 1.190 |
| | 26 27 | 13 | 1.146 | |
| | 27 28 | 72 | 1.863 | |
| | 28 29 | 37 | 1.580 | |
| | 29 30 | 43 | 1.644 | |
| 30 7 - 31 7 | 30 31 | 59 | 1.778 | 1.692 |
| | 31 7 - 1 8 | 55 | 1.748 | |
| | 1 8 2 8 | 50 | 1.708 | |
| | 2 3 / | | | |

Table 8, contd.

| Mid-date for Group | Individual Days | Daily Catch (n) | Log. Catch (Log. n + 1) | Mean Log. Catch for Group |
|---|---|---|---|---|
| | 2 8 - 3 8 | 75 | 1.881 | |
| | 3 4 | 689 | 2.839 | |
| 4 8 - 5 8 | 4 5 | 1152 | 3.062 | 2.909 |
| | 5 5 | 2841 | 3.454 | |
| | 6 7 | 2031 | 3.308 | |
| | 7 8 | 1207 | 3.082 | |
| | 8 9 | 30 | 1.491 | |
| 9 8 - 10 8 | 9 10 | 6185 | 3.791 | 2.868 |
| | 10 11 | 1426 | 3.154 | |
| | 11 12 | 659 | 2.820 | |
| | 12 13 | 482 | 2.683 | |
| | 13 14 | 54 | 1.740 | |
| 14 8 - 15 8 | 14 15 | 341 | 2.533 | 2.505 |
| | 15 16 | 195 | 2.292 | |
| | 16 17 | 1881 | 3.275 | |
| | 17 18 | 563 | 2.751 | |
| | 18 19 | 38 | 1.591 | |
| 19 8 | 19 20 | 61 | 1.792 | 1.812 |
| | 20 21 8 | 12 | 1.114 | |
| | 3 9 - 4 9 | 26 | 1.431 | |
| | 4 5 | 1136 | 3.056 | |
| 5 9 - 6 9 | 5 6 | 36 | 1.568 | 1.607 |
| | 6 7 | 5 | 0.778 | |
| | 7 8 | 15 | 1.204 | |
| | 8 9 | 10 | 1.041 | |
| | 9 10 | 66 | 1.826 | |
| 10 9 - 11 9 | 10 11 | 700 | 2.846 | 1.781 |
| | 11 12 | 118 | 2.076 | |
| | 12 13 | 12 | 1.114 | |
| | 13 14 | 1 | 0.301 | |
| | 14 15 | 0 | 0.000 | |
| 15 9 - 16 9 | 15 16 | 561 | 2.750 | 1.510 |
| | 16 17 | 250 | 2.400 | |
| | 17 18 | 124 | 2.097 | |
| | 18 19 | 51 | 1.716 | |
| | 19 20 | 0 | 0.000 | |
| 20 9 | 20 21 | 109 | 2.041 | 1.090 |
| | 21 22 9 | 3 | 0.602 | |

-o-o-

TABLE 9

Numbers of C. impunctatus caught by Traps A and B over a period of 24 hours in relation to Run of Wind for the same period.

| Date 1957 | Catch (n) | Log. (n + 1) | 5-day mean for Log. (n + 1), (M) | Difference as % M | Run of Wind (miles) |
|---|---|---|---|---|---|
| 2 7 | 61 | 1.792 | | | |
| 3 | 235 | 2.373 | | | |
| 4 | 360 | 2.558 | 2.318 | + 10.37 | 82.31 |
| 5 | 511 | 2.709 | 2.376 | + 14.01 | 90.32 |
| 6 | 142 | 2.155 | 2.407 | - 10.45 | 153.45 |
| 7 | 121 | 2.086 | 2.389 | - 12.64 | 209.85 |
| 8 | 335 | 2.526 | 2.308 | + 9.43 | 108.45 |
| 9 | 291 | 2.465 | 2.162 | + 14.05 | 140.93 |
| 10 | | | | | |
| 11 | 36 | 1.568 | 1.756 | - 10.67 | 211.99 |
| 12 | 38 | 1.591 | | | |
| 13 7 | 24 | 1.398 | | | |
| | | | | | |
| 19 7 | 29 | 1.477 | | | |
| 20 | 61 | 1.792 | | | |
| 21 | 14 | 1.176 | 1.502 | - 21.70 | 98.23 |
| 22 | 29 | 1.477 | 1.505 | - 1.85 | 115.85 |
| 23 | 36 | 1.588 | 1.408 | + 12.76 | 76.97 |
| 24 | 30 | 1.491 | 1.180 | + 26.41 | 138.00 |
| 25 | 1 | 0.301 | 1.247 | - 75.86 | 251.8 |
| 26 | 10 | 1.041 | 1.258 | - 17.21 | 351.8 |
| 27 | 64 | 1.813 | 1.296 | + 39.88 | 145.4 |
| 28 | 43 | 1.644 | 1.587 | + 3.57 | 135.9 |
| 29 | 47 | 1.681 | 1.733 | - 2.98 | 141.0 |
| 30 | 56 | 1.756 | 1.654 | + 6.16 | 73.7 |
| 31 7 | 58 | 1.771 | 1.735 | + 2.05 | 55.8 |
| 1 8 | 32 | 1.519 | 1.961 | - 22.58 | 184.9 |
| 2 | 88 | 1.949 | 2.214 | - 11.96 | ? |
| 3 | 648 | 2.812 | 2.515 | + 11.80 | 126.44 |
| 4 | 1048 | 3.021 | 2.883 | + 4.78 | 134.66 |
| 5 | 1883 | 3.275 | 3.111 | + 5.28 | 94.79 |
| 6 | 2275 | 3.357 | 3.008 | + 11.62 | 80.21 |
| 7 | 1225 | 3.089 | 3.159 | - 2.21 | 166.74 |
| 8 8/ | | | | | |

TABLE 9, contd.

| Date 1957 | | Catch (n) | Log. (n + 1) | 5-day mean for Log. (n + 1), (M) | Difference as % M. | Run of Wind (miles) |
|---|---|---|---|---|---|---|
| 8 | 8 | 197 | 2.297 | 3.132 | - 26.67 | 304.34 |
| 9 | | 5964 | 3.776 | 3.028 | + 24.71 | 86.09 |
| 10 | | 1389 | 3.143 | 2.950 | + 6.54 | 83.47 |
| 11 | | 681 | 2.834 | 2.864 | - 1.06 | 98.35 |
| 12 | | 500 | 2.700 | 2.615 | + 3.15 | 210.01 |
| 13 | | 73 | 1.869 | 2.426 | - 22.96 | 165.36 |
| 14 | | 336 | 2.528 | 2.499 | + 11.44 | 214.12 |
| 15 | | 158 | 2.201 | 2.536 | - 13.18 | 194.19 |
| 16 | | 1573 | 3.197 | 2.573 | + 24.24 | 115.00 |
| 17 | | 763 | 2.883 | 2.409 | + 19.67 | 106.32 |
| 18 | | 113 | 2.057 | 2.249 | - 8.52 | 216.78 |
| 19 | | 50 | 1.708 | | | |
| 20 | 8 | 24 | 1.398 | | | |
| 2 | 9 | 53 | 1.732 | | | 154.53 |
| 3 | | 24 | 1.398 | | | 60.43 |
| 4 | | 1007 | 3.003 | 1.709 | + 75.70 | 237.77 |
| 5 | | 42 | 1.634 | 1.585 | + 3.03 | 200.77 |
| 6 | | 5 | 0.778 | 1.535 | - 49.30 | 691.28 |
| 7 | | 12 | 1.114 | 1.300 | - 14.28 | 202.20 |
| 8 | | 13 | 1.146 | 1.537 | - 25.44 | 224.53 |
| 9 | | 66 | 1.826 | 1.820 | + 0.35 | 40.84 |
| 10 | | 663 | 2.822 | 1.826 | + 54.53 | 180.75 |
| 11 | | 154 | 2.190 | 1.657 | + 32.17 | 140.95 |
| 12 | | 13 | 1.146 | 1.293 | - 11.37 | 279.14 |
| 13 | | 1 | 0.301 | 1.269 | - 76.28 | 257.82 |
| 14 | | 0 | 0.000 | 1.316 | -100.00 | 256.76 |
| 15 | | 510 | 2.708 | 1.527 | + 77.33 | 155.72 |
| 16 | | 264 | 2.423 | 1.812 | + 33.73 | 76.74 |
| 17 | | 159 | 2.204 | 1.872 | + 17.72 | 38.45 |
| 18 | | 52 | 1.724 | 1.733 | - 0.50 | 102.23 |
| 19 | | 1 | 0.301 | 1.448 | - 79.22 | 96.11 |
| 20 | | 102 | 2.013 | | | |
| 21 | 9 | 9 | 1.000 | | | |

Degrees of freedom (n - 2) = 49
Correlation coefficient (r) = 0.384 (P 0.01)

Regression of Activity on Wind -0.13

TABLE 10 a

Numbers of C. impunctatus caught in relation to radiation intensity.

1 arbitrary unit = 1 mm. deflection of pen arm above base-line (mean deflection taken over hourly periods)

= 1.46 cal./cm.$^2$/hr.

| Class Number | Radiation Intensity: Arbitrary Units | Radiation Intensity: Cal./cm.$^2$/hr. | Total numbers caught by A and B. | Number of observations | Mean |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 8537 | 222 | 38.4 |
| 2 | 0.25 - 2.0 | 0.4 - 2.9 | 4407 | 162 | 27.2 |
| 3 | 3.0 - 5.0 | 4.4 - 7.3 | 3511 | 81 | 43.3 |
| 4 | 6.0 - 10.0 | 8.8 - 14.6 | 2267 | 87 | 26.1 |
| 5 | 11.0 - 15.0 | 16.1 - 21.9 | 2352 | 76 | 30.9 |
| 6 | 16.0 - 20.0 | 23.4 - 29.2 | 744 | 49 | 15.2 |
| 7 | 21.0 - 25.0 | 30.7 - 36.5 | 552 | 28 | 19.7 |
| 8 | 26.0 - 30.0 | 38.0 - 43.8 | 241 | 18 | 13.4 |
| 9 | 31.0 - 35.0 | 45.3 - 51.1 | 317 | 30 | 10.6 |
| 10 | 36.0 - 40.0 | 52.6 - 58.4 | 119 | 20 | 6.0 |
| 11 | 41.0 - 45.0 | 59.9 - 65.7 | 170 | 22 | 7.7 |
| 12 | 46.0 - 50.0 | 67.2 - 73.3 | 76 | 13 | 5.9 |
| 13 | 51.0 - 55.0 | 74.5 - 80.3 | 48 | 9 | 5.3 |

TABLE 10 b

| Class Number | Radiation Intensity Arbitrary Units | Cal./cm. /hr. | Sum of hourly catch for A and B as % daily catch. | Number of observations. | Mean |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 767.1 | 172 | 4.5 |
| 2 | 0.25 - 2.0 | 0.4 - 2.9 | 654.7 | 142 | 4.6 |
| 3 | 3.0 - 5.0 | 4.4 - 7.3 | 448.1 | 80 | 5.6 |
| 4 | 6.0 - 10.0 | 8.8 - 14.6 | 327.1 | 71 | 4.6 |
| 5 | 11.0 - 15.0 | 16.1 - 21.9 | 335.6 | 74 | 4.5 |
| 6 | 16.0 - 20.0 | 23.4 - 29.2 | 94.0 | 41 | 2.3 |
| 7 | 21.0 - 25.0 | 30.7 - 36.5 | 60.6 | 21 | 2.9 |
| 8 | 26.0 - 30.0 | 38.0 - 43.8 | 43.4 | 16 | 2.7 |
| 9 | 31.0 - 35.0 | 45.3 - 51.1 | 46.9 | 22 | 2.1 |
| 10 | 36.0 - 40.0 | 52.6 - 58.4 | 30.9 | 18 | 1.7 |
| 11 | 41.0 - 45.0 | 59.9 - 65.7 | 37.5 | 17 | 2.2 |
| 12 | 46.0 - 50.0 | 67.2 - 73.0 | 15.6 | 11 | 1.4 |
| 13 | 51.0 - 55.0 | 74.5 - 80.3 | 13.7 | 7 | 2.0 |

TABLE 10 c

Analysis of Variance for 13 Classes.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 691 | 17,500.04 | |
| Between Classes | 12 | 824.7 | 68.7 |
| Within Classes | 679 | 16,675.34 | 24.56 |

Variance Ratio = 2.79 ($P < 0.1$ %) Significant.

. . .

Analysis of Variance for Classes 1 and 2.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 313 | 4,753.63 | |
| Between Classes | 1 | 1.7 | 1.7 |
| Within Classes | 312 | 4.751.9 | 15.23 |

Variance Ratio = 0.01 ($P > 5.0$ %) Insignificant.

Analysis of Variance for Classes 1, 2 and 3.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 393 | 7,186.01 | |
| Between Classes | 2 | 75.1 | 37.5 |
| Within Classes | 391 | 7,110.9 | 18.2 |

Variance Ratio = 2.10 ($P > 5.0$ %) Insignificant.

Analysis of Variance for Classes 1, 2, 3 and 4.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 464 | 13,619.7 | |
| Between Classes | 3 | 76.2 | 25.4 |
| Within Classes | 461 | 13,543.5 | 29.4 |

Variance Ratio = 0.86 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 1, 2, 3, 4 and 5.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 538 | 15,929.0 | |
| Between Classes | 4 | 78.4 | 19.6 |
| Within Classes | 534 | 15,850.6 | 29.7 |

Variance Ratio = 0.65 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 1, 2, 3, 4, 5 and 6.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 579 | 16,401.61 | |
| Between Classes | 5 | 299.0 | 59.8 |
| Within Classes | 574 | 16,102.61 | 28.05 |

Variance Ratio = 2.13 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 1, 2, 3, 4, 5, 6 and 7.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 600 | 16,600.29 | |
| Between Classes | 6 | 353.65 | 58.94 |
| Within Classes | 594 | 16,246,64 | 27.35 |

Variance Ratio = 2.155 (P < 5.0 %) Significant.

. . .

Analysis of Variance for Classes 12 and 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 17 | 47.0 | |
| Between Classes | 1 | 1.2 | 1.2 |
| Within Classes | 16 | 45.8 | 2.9 |

Variance Ratio = 0.41 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 11, 12 and 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 34 | 135.9 | |
| Between Classes | 2 | 4.1 | 2.0 |
| Within Classes | 32 | 131.8 | 4.1 |

Variance Ratio = 0.48 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 10, 11, 12 and 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 52 | 189.6 | |
| Between Classes | 3 | 4.5 | 1.5 |
| Within Classes | 49 | 185.1 | 3.8 |

Variance Ratio = 0.39 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 9, 10, 11, 12, 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 74 | 264.3 | |
| Between Classes | 4 | 20.2 | 5.05 |
| Within Classes | 70 | 244.1 | 3.5 |

Variance Ratio = 1.44 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 8, 9, 10, 11, 12 and 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 90 | 452.6 | |
| Between Classes | 5 | 13.9 | 2.8 |
| Within Classes | 85 | 438.7 | 5.2 |

Variance Ratio = 0.54 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 7, 8, 9, 10, 11, 12 and 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 111 | 607.96 | |
| Between Classes | 6 | 25.3 | 4.2 |
| Within Classes | 105 | 582.66 | 5.5 |

Variance Ratio = 0.76 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 6, 7, 8, 9, 10, 11, 12, 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 152 | 860.2 | |
| Between Classes | 7 | 25.5 | 3.6 |
| Within Classes | 145 | 834.7 | 5.7 |

Variance Ratio = 0.63 (P > 5.0 %) Insignificant.

Analysis of Variance for Classes 5, 6, 7, 8, 9, 10, 11, 12, 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 226 | 3,430.09 | |
| Between Classes | 8 | 288.27 | 36.0 |
| Within Classes | 218 | 3.141.82 | 14.4 |

Variance Ratio = 2.50 (P = 1 %) Significant.

. . .

Analysis of Variance for 2 Groups: Classes 1 - 6 and 7 - 13.

| Source of Variance | D. f. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 691 | 17,500.04 | |
| Between Groups | 1 | 457.24 | 457.24 |
| Within Groups | 690 | 17,042.8 | 24.69 |

Variance Ratio = 18.52 ($P < 0.1\ \%$) Significant.

---